

Use of Liposomal Gentamicin for Treatment of 5 Foals with Experimentally Induced *Rhodococcus equi* Pneumonia

N.D. Cohen, S. Giguère, A.J. Burton, J.N. Rocha, L.J. Berghaus, C.N. Brake, A.I. Bordin, and M.C. Coleman

Background: Adverse effects of, and bacterial resistance to, macrolides used to treat *Rhodococcus equi* infections have prompted search for clinically effective alternative antimicrobials. Liposomal gentamicin (LG) is effective against *R. equi* in vitro and decreases tissue concentrations of *R. equi* in experimentally infected mice. Effectiveness of LG treatment of foals with *R. equi* pneumonia, however, has not been described.

Hypothesis: Liposomal gentamicin is safe and effective for treating foals with *R. equi* pneumonia.

Animals: Ten foals with experimentally induced *R. equi* pneumonia.

Methods: Pilot treatment trial. Foals with pneumonia induced by intrabronchial instillation of *R. equi* were randomly allocated to receive either clarithromycin combined with rifampin (CLR + RIF) PO or LG IV, and followed by daily physical examinations and weekly thoracic ultrasonography and serum creatinine concentration determinations until the resolution of clinical signs. Treatment success was defined as the resolution of clinical signs and ultrasonographically identified pulmonary abscesses.

Results: All 10 foals were successfully treated. Two of 5 foals treated with LG developed azotemia within 1 week; LG was discontinued and treatment switched to CLR + RIF for these foals. None of the CLR + RIF treated foals developed azotemia.

Conclusions and Clinical Importance: Liposomal gentamicin IV can be effective for treatment of *R. equi* pneumonia, but nephrotoxicity indicates that an alternative dosing interval or route (such as nebulization) will be needed before LG is adequately safe for clinical use. Larger comparative trials will be needed to evaluate the relative efficacy of a safer LG dosage regimen.

Key words: Adverse effects; Antibiotic choices; Macrolides; Nephrotoxicity.

Pneumonia caused by the facultative intracellular pathogen *Rhodococcus equi* is an important cause of disease and death in foals worldwide.¹ For approximately 30 years, the combination of a macrolide antibiotic and rifampin (RIF) has been the treatment of choice.¹ Despite long-standing use, there is need for alternative antimicrobials to this combination. The mortality rate of severely affected foals is approximately 30%,^{1,2} indicating that more effective treatment would be clinically useful. Adverse effects of macrolides (including diarrhea and hyperthermia) can be common and potentially life-threatening.^{1,3} Moreover, evidence

Abbreviations:

BAL	bronchoalveolar lavage
CLR	clarithromycin
IV	intravenous
LG	liposomal gentamicin
PO	per os
RIF	rifampin
TUS	thoracic ultrasonography
TBA	tracheobronchial aspirate

exists that resistance to macrolides is emerging and can worsen prognosis for affected foals.⁴ Macrolide-resistant isolates may be isolated from foals before treatment or during the course of treatment.⁴ Gentamicin has bactericidal activity against virulent *R. equi* in vitro,^{4,5} but reportedly has poor clinical efficacy that has been attributed to poor intracellular penetration.¹ Studies have shown that, compared with the conventional formulation of free gentamicin, liposomal gentamicin (LG) has significantly enhanced cellular penetration and activity against facultative intracellular bacteria such as *Listeria monocytogenes*, *Mycobacterium avium*, and *Salmonella* spp., both in vitro and in vivo.^{6,7} Recently, a formulation of LG has been developed for use in foals (patent pending).^{8,9,a} This formulation was administered to 8 healthy foals and was well tolerated with no evidence of alterations in renal function and similar results for indices of renal injury (eg, ratios of either urinary γ -glutamyltransferase or urinary protein to urinary creatinine, fractional clearance of electrolytes) to those observed after administration of free gentamicin. Moreover, LG had a significantly longer half-life, larger

From the Department of Large Animal Clinical Sciences, Texas A&M University, College Station, TX (Cohen, Rocha, Brake, Bordin, Coleman); and the Department of Large Animal Medicine, College of Veterinary Medicine, University of Georgia, Athens, GA (Giguère, Burton, Berghaus)

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Corresponding author: N.D. Cohen, Department of Large Animal Clinical Sciences, Texas A&M University, 4475 TAMU, College Station, TX 77843-4475; e-mail: ncohen@cvm.tamu.edu.

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volume of distribution, and higher concentration in bronchoalveolar lavage (BAL) cells than free gentamicin.⁸ Relative to combination of clarithromycin (CLR) and RIF, LG is approximately 20 times more effective at killing *R. equi* in infected macrophages, and significantly more effective at decreasing tissue concentrations of *R. equi* in experimentally infected mice.⁹ Collectively, these data indicate that LG might be an effective alternative to standard treatment of foals with *R. equi* pneumonia. Thus, we investigated the response to LG in foals experimentally infected intrabronchially with *R. equi*.

Materials and Methods

The protocol for this project was approved by Texas A&M University’s Animal Care and Use Committee (2013-0171). Ten healthy Quarter Horse foals at Texas A&M University were studied. The sample size for this pilot study was determined by convenience, using foals available from a separate study of *R. equi* infection and immunity during the months of March through August, 2014. Foals were randomly assigned to treatment with either LG IV (6.6 mg/kg IV q24h) or CLR + RIF PO (CLR: 7.5 mg/kg PO q12h; RIF: 5 mg/kg PO q12h) using an allocation ratio of 1, a computerized^b random number generator to generate a random sequence of 0s and 1s, with a value of 0 for CLR + RIF and 1 for LG assigned by coin flip (using tails = LG), and group assignment completed for a given group when 5 foals were assigned to that group. The LG was prepared as previously reported.⁵ The dosage and dosing interval for LG were selected based on a prior study demonstrating peak concentrations of gentamicin in bronchoalveolar lavage cells approximately 10 times above the MIC₉₀ of *R. equi*.⁸ At approximately 21 days of age (range 20–25 days), each foal underwent physical examination, thoracic ultrasonography (TUS), CBC, and serum creatinine concentration determination, and then was infected transendoscopically with a 40-mL suspension of virulent *R. equi* containing approximately 1 × 10⁶ viable bacteria, with 20 mL infused into the right mainstem bronchus and 20 mL into the left mainstem bronchus.

After intrabronchial infection, each foal was inspected daily to monitor the following variables: (1) rectal temperature, heart rate, and respiratory rate; (2) tracheal and thoracic auscultation; (3) coughing; (4) nasal discharge; (5) respiratory effort; and, (6) lethargy, including posture and frequency of suckling. Foals also had weekly TUS examinations to monitor lung lesions and weekly measurements of serum creatinine concentration to monitor for evidence of renal disease. When foals developed clinical signs of pneumonia (rectal temperature > 39.7°C [103.5°F]), coughing, respiratory rate >40 breaths/min, lethargy, and subjective assessment

of increased respiratory effort), a transendoscopic tracheobronchial aspirate (TBA) was collected, using a sterile, triple-lumen, double-guarded catheter system^c and 20 mL of sterile 0.9% physiological saline solution, and submitted for cytology and microbiologic culture. After the TBA was collected, foals were treated with their assigned treatment. For LG, an over-the-wire IV catheter was placed,^d and catheters were flushed with 0.9% NaCl containing 10 IU/mL of heparin 3 times daily to ensure patency. After infection, foals were housed with their dams in individual pens with shelter. After diagnosis of pneumonia, foals continued to be monitored with twice-daily physical examinations, weekly TUS and serum creatinine concentrations, and biweekly CBC through resolution of clinical signs and thoracic lesions. Continuous variables were compared between groups using a Wilcoxon rank-sum test and categorical variables were compared with Fisher’s exact tests.^b A significance level of *P* < .05 was used for analyses.

Results

Foals in the 2 treatment groups appeared similar regarding age at infection, onset of clinical signs, age of onset of lesions detected by TUS, size of TUS lesions when first detected, and serum creatinine concentration (Table 1). After 1 week of treatment, however, 2 LG foals developed azotemia and were switched to CLR + RIF treatment. The maximum serum creatinine concentrations and times to maximum concentration were 4.02 and 6.74 mg/dL at 12 and 7 days after initiating LG treatment, respectively. Azotemia resolved in both foals, after 79 and 17 days, respectively. Results of urinalysis were within reference ranges at the time of peak azotemia and after return to within reference range of serum creatinine concentration. Both azotemic foals were each treated for 7 days with LG and subsequently for 22 or 34 days with CLR + RIF. Azotemia was managed with IV fluids involving bolus administration of 25 mL/kg of 0.9% sodium chloride q8h for 4 days to each foal. Both foals appeared somewhat lethargic but continued to suckle well. These 2 foals were excluded from comparisons of LG and CLR + RIF treatment.

Clinical outcomes appeared similar except for the duration of cough among the 3 foals treated with LG and the 5 foals treated with CLR + RIF (Table 2). All foals developed fever (>39.7°C), cough, tachypnea (rate > 40 breaths/min), TUS lesions, and had cytologic evidence of sepsis in, and virulent *R. equi* isolated from, TBA fluid. The duration of antimicrobial treatment and

Table 1. Baseline characteristics of foals in the 2 treatment groups (clarithromycin + rifampin [CLR + RIF] or liposomal genatamicin [LG]) did not differ significantly. Data are reported as medians (range).

Variable	CLR + RIF (N = 5)	LG (N = 5)	<i>P</i> Value*
Age infected (days)	21 (20–23)	23 (21–25)	.1666
Age at onset of coughing (days)	29 (27–39)	36 (31–39)	.4005
Age at onset of fever (days)	34 (30–37)	36 (34–37)	.2017
Age at onset of tachypnea (days)	24 (20–35)	23 (21–25)	.9163
Age at onset of ultrasonographic lesion of maximal diameter ≥ 1 cm (days)	37 (33–39)	39 (36–46)	.1679
Maximal diameter of ultrasonographic lesions at onset (cm)	6.0 (3.5–26.4)	8.5 (3.4–19.2)	.8413
Serum creatinine concentration (mg/dL)	1.1 (0.8–1.4)	1.1 (0.7–1.3)	.9871

**P* values derived by Wilcoxon rank-sum test.

Table 2. Clinical signs and thoracic ultrasonographic findings in 8 foals experimentally infected with virulent *Rhodococcus equi* treated either with clarithromycin and rifampin combination (CLR + RIF) or liposomal gentamicin (LG). These data exclude 2 foals who were switched from treatment with LG to CLR + RIF as a result of development of azotemia during treatment with LG (see text for details). Data are reported as medians (ranges).

Variable	CLR + RIF (N = 5)	LG (N = 3)	P Value*
Age infected (days)	21 (20–23)	22 (21–24)	.4478
Age at onset of coughing (days)	29 (27–39)	36 (31–39)	.4534
Duration of coughing (days)	8 (4–13)	19 (12–20)	.0714
Age at onset of fever (days)	34 (30–37)	36 (34–42)	.3653
Duration of fever (days)	6 (2–9)	8 (6–17)	.2938
Age at onset of tachypnea (days)	24 (20–35)	23 (22–25)	.8808
Duration of tachypnea (days)	35 (27–41)	41 (35–58)	.1970
Age at onset of ultrasonographic lesion of maximal diameter \geq 1 cm (days)	37 (33–39)	39 (39–46)	.1847
Duration of ultrasonographic lesion of score \geq 2 (days)	20 (7–30)	15 (14–42)	.7857
Maximum cumulative diameter of lesions (cm)	19.9 (3.0–26.4)	19.2 (9.6–36.0)	.7368
Duration of antimicrobial treatment (days)	25 (12–35)	24 (13–41)	.8808

*P values derived by Wilcoxon rank-sum test.

duration of TUS lesions were similar for foals in both groups. The results of these statistical comparisons of clinical outcomes should be interpreted cautiously because of the low statistical power of this pilot study. Larger scale studies will be necessary to assess the relative effectiveness of LG delivered by a safer route of administration or dosing regimen.

Diarrhea, defined as liquid feces that formed a liquid pool on the ground, developed in 7 of the 10 foals included in the study: 4 of the 5 foals in the CLR + RIF group, 2 foals after being switched from LG to CLR + RIF, and 1 foal in the LG group. Diarrhea was short-lived in all foals (median 3 days; range 1–8 days) and was managed without discontinuing antimicrobial treatment. The 6 foals that developed diarrhea while receiving CLR + RIF developed diarrhea within 72 h of initiating treatment; diarrhea developed in the foal in the LG group 3 days after LG treatment was initiated. Although more foals treated with CLR + RIF (86%; 6/7) developed diarrhea than did LG-treated foals (33%; 1/3), this difference was not significant ($P = .1883$; Fisher's exact test). As noted above, the statistical power of this pilot study was limited and diarrhea is a common complication of treatment of foals with macrolides.³ It is thus plausible that LG might be less likely to result in diarrhea than macrolides.

Discussion

Results of the study indicate that LG IV can be effective for treatment of *R. equi* pneumonia, but evidence of nephrotoxicity indicates that an alternative dosing interval or route (eg, nebulization) will be needed before LG is adequately safe for widespread use. Because of the paucity of clinically effective alternatives to macrolides and RIF for treating *R. equi* pneumonia,¹ the emergence of resistance of *R. equi* to macrolides,⁴ and adverse effects of macrolides (eg, diarrhea),^{1,3} the finding that LG is effective for treating foals with *R. equi* pneumonia is clinically important. The high incidence of azotemia after only 1 week of treatment was not

expected because serum creatinine concentration did not increase after 7 daily IV doses to healthy foals aged 5–7 weeks in a prior study.⁸ To the authors' knowledge, nephrotoxicity with LG has not been thoroughly investigated in other species. To determine the nephrotoxicity of LG relative to free gentamicin, it would have been necessary to have included a group of foals treated with free gentamicin. Experimental results in foals indicate that LG and free gentamicin result in similar indices of renal injury.⁸ Ill foals may not have nursed as well as healthy foals, potentially predisposing them to dehydration and nephrotoxicity. Ill foals also might have lost more body fluid by sweating from fever and greater respiratory effort. Moreover, 1 of the azotemic foals received flunixin meglumine (1.1 mg/kg PO q12h for 3 days) for management of fever and lethargy concurrently with LG which might have predisposed to nephrotoxicity. Conceivably, different formulations or a longer dosing interval might reduce the incidence of nephrotoxicity. The elimination half-life of LG is significantly longer than that of free gentamicin, and concentrations of LG in BAL fluid cells 48 h after dosing still are above the MIC of the drug against *R. equi*.⁹ Therefore, administration q48h might be therapeutically sufficient while minimizing the risk of nephrotoxicity. Alternatively, LG delivered topically to the lungs by nebulization might be effective. In a prior study, administration of LG by nebulization resulted in minimal systemic absorption with similar concentrations in BAL cells and higher concentrations in pulmonary epithelial lining fluid compared to IV administration.⁹

This pilot study had a number of limitations. First and foremost, the small sample size greatly limited statistical power. Clearly, larger scale therapeutic trials are needed to characterize the effectiveness and safety of LG. Nonetheless, given the paucity of effective alternatives and the emergence of resistance to macrolides, our observation that LG can be used effectively to treat foals with *R. equi* lends support to the rationale of conducting such studies, once a safer dosage interval or route of administration for foals is established. Second,

those treating and assessing the foals (AIB, CNB, MCC, NDC, JR) were not blinded to treatment status. Third, no placebo control group was included. Evidence exists that many foals with subclinical pneumonia or mild to moderate pneumonia attributed to *R. equi* will recover without treatment, but treatment with macrolides also has been demonstrated to be superior to placebo.¹⁰ Despite these limitations, results of this study should be of interest to veterinarians who treat foals with *R. equi* pneumonia.

Footnotes

- ^a Patent pending with the University of Georgia Research Foundation, Inc. as the assignee. One author (S. G.) is named as co-inventor; S. G. was not involved in the clinical evaluation of foals.
- ^b S-PLUS (Version 8.2), TIBCO, Inc., Seattle, WA
- ^c Triple stage tracheal wash catheter, MILA International Inc., Erlanger, KY
- ^d Long-term MILACATH kit with guidewire, MILA International Inc., Erlanger, KY
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Conflict of Interest Declaration: The University of Georgia Research Foundation, Inc. is the assignee of pending patents on the formulation described in the manuscript. One author (Steeve Giguere) is named as co-inventor on those patent applications but he was not involved in the evaluation of the foals and assessment of clinical efficacy.

Off-label Antimicrobial Declaration: Authors declare no off-label use of antimicrobials.

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