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Gallium maltolate: safety in neonatal foals following multiple enteral administrations

R. J. MARTENS*
N. D. COHEN*
V. R. FAJT†
J. R. NERREN*
M. K. CHAFFIN*
R. J. TAYLOR‡ &
L. R. BERNSTEIN§

**Department of Large Animal Clinical Sciences; †Department of Veterinary Physiology and Pharmacology; ‡Department of Veterinary Integrative Biosciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX; §Terrametrix (Bernstein), 285 Willow Rd., Menlo Park, CA, USA*

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Ronald J. Martens, Department of Large Animal Clinical Sciences, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, College Station, TX 77843, USA. E-mail: rmartens@cvm.tamu.edu; Fax: (979) 847-8863

INTRODUCTION

Gallium maltolate (GaM) has been proposed as a new agent for the prevention and control of foal pneumonia caused by the intracellular bacterium *Rhodococcus equi*. This proposal is based on the observed antimicrobial effectiveness of GaM on *R. equi* (Harrington. *et al.*, 2006; Martens *et al.*, 2007a), and the known pharmacokinetics of GaM in neonatal foals (Martens *et al.*, 2007b). The use of GaM represents a novel approach to combating infections: the exploitation of a major stress already imposed on an organism by the host's environment. Adequate availability of ferric iron (Fe^{3+}) is essential for the growth of most pathogenic bacteria, including *R. equi* (Jordan *et al.*, 2003), which rely on Fe^{3+}-dependent enzyme systems (Bullen *et al.*, 2005). A major host innate antimicrobial defense mechanism is the sequestration of available Fe^{3+} by host plasma proteins, primarily transferrin and lactoferrin. *Rhodococcus equi*, however, can acquire and utilize transferrin- and lactoferrin-bound iron, thereby circumventing this defense mechanism (Jordan *et al.*, 2003).

Gallium, a trivalent semi-metal that shares many chemical similarities with Fe^{3+} and functions as a ferric iron mimic, has been used to control various microorganisms by exploiting their iron dependency (Bernstein, 1998; Oyebode *et al.*, 2000; Harrington *et al.*, 2006; Kaneko *et al.*, 2007). Gallium competes with Fe^{3+} for uptake by bacteria and is incorporated into crucial Fe^{3+}-dependent enzyme systems. Trivalent gallium, unlike Fe^{3+}, is unable to undergo redox-cycling, which leads to inactivation of some Fe^{3+}-dependent enzymes (particularly ribonucleotide reductase), causing bacterial stasis and death (Bernstein, 1998).

Gallium salts (e.g., gallium chloride, gallium nitrate) are not well absorbed orally (Bernstein, 1998). In the only reports of an herbivore species or a neonate in which Ga administration has been assessed, neonatal calves treated with oral gallium chloride developed severe intestinal lesions, alterations in enteric microflora, and diarrhea (Fettman *et al.*, 1987a,b). A citrate-chelated gallium nitrate solution for injection (Ganite®, Genta Inc., Berkeley Heights, NJ, USA), which is FDA-approved for treatment of human cancer-related hypercalcemia, is associated with renal toxicity when administered as an IV bolus (Bernstein, 2005).

Gallium maltolate, a coordination complex of Ga and maltol, developed by a co-author (LRB), has high oral bioavailability in humans and a variety of other species (Bernstein *et al.*, 2000), and attains potentially therapeutic serum concentrations in neonatal foals (Martens *et al.*, 2007b). Adverse responses to enteral GaM have not been reported in humans or laboratory animal species, but safety studies have not been reported for either neonates or horses.

During the neonatal period (first days-to-weeks of life) most mammals have immature or inefficient organ systems and are undergoing a variety of physiologic adaptations that can impact their pharmacologic and toxicologic responsiveness (Koterba, 1990). This report assesses the safety of GaM in neonatal foals by comparing clinical and clinicopathologic parameters of foals that received five daily doses of enteral GaM or distilled water. The authors believe that this is the first report on the safety of GaM therapy in neonates of any species.

Rhodococcus equi is a facultative intracellular bacterium that causes severe bronchopneumonia in foals and immunocompromised people (Giguere & Prescott, 1997). Most foals appear to become infected within the first few days of life (Horowitz *et al.*, 2001), when they may have immature or ineffective immune responses (Boyd *et al.*, 2003; Chaffin *et al.*, 2004). Because the

Table 1. Mean serum gallium concentrations in six neonatal foals at 0 h, 2 h after each of five daily treatments with intragastric gallium maltolate (20 mg/kg), and at 48 h after the final treatment (144 h)

	Serum conc. (ng/mL)	
Time (h)	Mean	SD
0	0.96	0.12
2	1089	575
26	1524	793
50	1369	399
74	920	240
98	739	419
144	212	53

proposed GaM chemotherapy strategy would entail oral administration to young foals, this study was conducted to assess the safety of enteral GaM in neonatal foals by comparing clinical, hematologic, and serum biochemical parameters between foals treated with GaM or distilled water (controls).

Nine 1- to 2-day-old Quarter Horse foals (five male, four female), weighing 39 to 59 kg (median = 47 kg) were studied. Each foal received adequate transfer of maternal antibodies (serum IgG ≥ 800 mg/dL) as determined by a commercial assay (Snap Test, IDEXX Laboratories, Westbrook, ME, USA). Foals were deemed healthy for inclusion in the study based on absence of abnormal findings on physical examination, complete blood count (CBC), and serum biochemical profile. Each foal was housed in a box stall with its dam, and allowed to nurse *ad libitum*.

At 1 to 2 days of age, six principal (GaM) foals (four male, two female) were administered a distilled water solution of GaM (10 mg/mL), via naso-gastric tube, at 20 mg/kg, q 24 h, for 5 days. Three 1- to 2-day- old control foals (one male, two female), were administered distilled water via naso-gastric tube at a weight-based volume identical to that used in GaM foals, and at the same time intervals. Foals were weighed daily prior to treatment to determine GaM dosages.

All foals were monitored daily, beginning immediately prior to the first treatment (0 h) and extending until 48 h after the final treatment (144 h). Clinical monitoring consisted of rectal temperature, body weight, and clinical scoring of physical activity, fecal consistency, and respiratory effort (Appendix 1). In addition, CBCs, serum biochemical profiles, serum total iron-binding capacity (TIBC), and total serum iron concentrations were monitored, and transferrin saturation percentages calculated. Clinicopathologic assays were conducted by the Clinical Pathology Laboratory, College of Veterinary Medicine and Biomedical Sciences, Texas A&M University, and the study was approved by the Texas A&M University Institutional Animal Care and Use Committee.

Serum gallium concentrations were assessed in GaM foals to verify GaM absorption. Blood samples were collected via jugular venipuncture prior to GaM treatment, at 2 h post-treatment on each of five consecutive days and at 144 h. Serum was harvested and stored at −80 °C, and all samples assayed for Ga at the same time. Gallium concentrations were measured by inductively coupled plasma/mass spectrometry (ICP/MS) as previously described (Martens *et al.*, 2007b). Post-treatment serum Ga concentrations in GaM foals were consistent with those previously reported (Martens *et al.*, 2007b) (Table 1).

Values of clinical, hematological, and serum biochemical parameters were compared between GaM and control foals at 0 and at 144 h. In addition, the paired differences in values between 0 and 144 h for these parameters were compared between GaM and control foals. Comparisons were made using the Wilcoxon rank-sum test, using a significance level of $P < 0.05$; absence of statistically significant findings obviated an adjustment for multiple comparisons.

In this study, daily parameter assessments were conducted in an effort to identify and characterize prodromal signs of toxicosis. Because there were no apparent aberrations in any of the parameters evaluated throughout the study period, we elected to compare all parameters of the GaM and control groups at 0 and 144 h (Tables 2, 3, & 4), and the paired differences between groups at 0 and 144 h. There were no significant differences in values between groups, or in paired differences in values between groups, at 0 and 144 h.

Considering all foals together (i.e., ignoring any effects of treatment), there were significant decreases between 0 and 144 h in activity of serum alkaline phosphatase and concentrations of blood urea nitrogen, creatinine, globulin, hemoglobin, magnesium, packed cell volume, total protein, and iron. Additionally, there were increases between 0 and 144 h in concentration of total white blood cells and neutrophils. Observed ranges and

Table 2. Comparative data on clinical variables in neonatal foals at 0 h [prior to enteral treatment of Principal foals with GaM (20 mg/kg BW) and enteral treatment of Control foals with distilled water], and at 144 h (2 days after the last of five daily treatments with GaM or distilled water)

	0 h					144 h				
	GaM		Control			GaM		Control		
Variable	Median	Range	Median	Range	*P*-value	Median	Range	Median	Range	*P*-value
Body wt. (kg)	49	46 to 59	43	39 to 45	0.124	58	54 to 67	53	48 to 54	0.138
Temperature (°F)	101.8	100.0 to 102.8	102	101.8 to 102.2	0.696	101.5	100.8 to 101.8	101.2	101.0 to 102.3	0.895
*Physical activity score	0	0	0	0	NA	0	0	0	0	NA
*Fecal consistency score	1	1	1	1	NA	0.5	0 to 1	0	0 to 1	0.766
*Respiratory effort score	0	0	0	0	NA	0	0	0	0	NA

*Clinical scoring system – see Appendix 1.

Table 3. Comparative data on hematologic variables in neonatal foals at 0 h [prior to enteral treatment of Principal foals with GaM (20 mg/kg BW) and Control foals with distilled water], and at 144 h (2 days after the last of five daily treatments with GaM or distilled water)

	0 h					144 h				
	GaM		Control			GaM		Control		
Variable	Median	Range	Median	Range	*P*-value	Median	Range	Median	Range	*P*-value
WBC ($\times 10^3/\mu L$)	7.4	2.8 to 11.8	6.7	4.8 to 9.3	0.905	9.85	8.2 to 12.8	8.7	6.3 to 10.1	0.243
RBC ($\times 10^3/\mu L$)	11.2	9.3 to 13.2	10.3	10.1 to 11.3	0.437	9.6	8.3 to 12.0	8.6	7.9 to 9.5	0.167
Hgb (g/dL)	14.8	12.6 to 17.5	13.5	13.4 to 14.8	0.381	12.5	11.2 to 16.5	11.6	10.3 to 12.5	0.262
PCV (%)	42.3	36.9 to 49.5	41	39.7 to 43.6	0.897	34.9	32.1 to 46.6	33.1	35.1 to 39.9	0.262
MCV (fl)	38	35.4 to 40.6	38.9	38.7 to 39.3	0.548	37	34.8 to 40.1	37.7	28.3 to 37.1	0.905
MCHC (g/dL)	34.8	34.2 to 35.3	33.9	33.8 to 34.0	0.2075	35.2	34.2 to 36.4	34.9	34.6 to 35.7	0.696
Plasma protein (g/dL)	6.6	4.8 to 7.3	6.1	5.4 to 6.5	0.548	6.1	5.0 to 6.7	5.7	5.4 to 5.8	0.547
Fibrinogen (mg/dL)	200	100 to 500	300	50 to 300	0.895	200	10 to 300	200	200 to 300	0.391
Neutrophils (total)	6128	1,400 to 9,322	5026	3,360 to 6,975	0.714	7777	5,986 to 9,984	6,525	4,851 to 7,676	0.262
Neutrophils (%)	76.5	50 to 89	75	70 to 75	0.897	78	73 to 90	76	75 to 77	0.435
Lymphocytes (total)	1350	870 to 2,242	1473	1,200 to 2,139	0.697	1,877	824 to 2,688	1,740	197 to 2,222	0.714
Lymphocytes (%)	22	10.0 to 42.0	23	22.0 to 25.0	1	20	8 to 24	20	19 to 22	1
Monocytes (total)	102.5	0 to 196	96	67.0 to 186	0.905	146	0 to 412	202	87 to 252	0.905
Monocytes (%)	1	0 to 6.0	2	1 to 3	0.583	1.5	0 to 6.0	2	1.0 to 4.0	0.591

Table 4. Comparative data on serum biochemical variables in neonatal foals at 0 h [prior to enteral treatment of GaM foals with GaM (20 mg/kg BW) and Control foals with distilled water], and at 144 h (2 days after the last of five daily treatments with GaM or distilled water)

	0 h					144 h				
	GaM		Control			GaM		Control		
Variable	Median	Range	Median	Range	*P*-value	Median	Range	Median	Range	*P*-value
Glucose (mg/dL)	167	146.0 to 206.0	137	134 to 164	0.167	136	117 to 158	160	116 to 183	0.517
BUN (mg/dL)	10	5.0 to 19.0	8	6.0 to 14.0	1	4	2.0 to 6.0	4	4	0.759
Creatinine (mg/dL)	1.6	1.4 to 1.8	1.5	1.2 to 1.7	0.361	1.2	1.1 to 1.4	1.3	1.1 to 1.3	1
Magnesium (mg/dL)	2.4	2.2 to 2.8	2.4	2.3 to 2.5	0.794	2	1.8 to 2.3	2	2.0 to 2.3	0.791
Calcium (mg/dL)	10.8	10.2 to 11.7	10.9	10.9 to 11.2	0.696	11.3	10.9 to 11.9	11.5	11.3 to 11.5	0.362
Phosphorus (mg/dL)	5.8	4.9 to 6.9	6.1	5.3 to 6.5	1	7.4	6.7 to 8.5	7.6	6.9 to 7.9	0.795
Total protein (g/dL)	6.3	4.2 to 6.7	5.6	4.9 to 5.8	0.364	5.7	4.0 to 6.1	5.1	4.6 to 5.2	0.437
Albumen (g/dL)	2.3	2.2 to 2.6	2.1	2.0 to 2.2	0.245	2.2	2.1 to 2.3	1.9	1.9 to 2.1	0.246
Globulin (g/dL)	4	2.2 to 4.2	3.3	3.0 to 3.7	0.548	3.5	1.9 to 3.8	3	2.7 to 3.3	0.548
AST (U/L)	175	128 to 188	152	150 to 334	0.905	298	230 to 374	287	3.4 to 699.0	0.905
CPK (U/L)	146	99 to 840	127	117 to 162	1	142.5	94 to 373	111	92 to 144	0.381
Alkaline phosphatase (U/L)	1,612.5	962 to 1,936	2042	1,407 to 2,456	0.381	989.5	783 to 118	971	793 to 1,752	0.905
GGT (U/L)	43	35 to 64	45	40.0 to 93.0	0.604	65	39 to 199	61	60 to 620	0.795
Total bilirubin (mg/dL)	2.8	2.0 to 5.6	1.8	1.7 to 3.0	0.138	2	2.0 to 5.6	1.7	1.5 to 1.8	0.1521
Serum iron (μg/dL)	244.5	117 to 284	166	94 to 251	0.381	135.5	53 to 196	124	53 to 178	0.604
TIBC (μg/dL)	397	306 to 484	407	355 to 436	0.805	432.5	406 to 497	427	408 to 469	1
Transferrin saturation (%)	55	38.0 to 71	47	23 to 58	0.548	31.5	13 to 48	30	12 to 38	0.548

temporal changes in hematologic and serum chemistry profiles of both groups, regardless of treatment, were anticipated based on previous reports (Bauer, 1990; Harvey, 1990).

Neither gastrointestinal nor renal toxicities have been associated with GaM therapy (Bernstein *et al.*, 2000), and in this study fecal consistency as well as serum BUN and creatinine concentrations were similar between groups. In addition, the rate of weight gain was similar between groups and within the normal range (Koterba, 1990).

Concerns are oftentimes expressed about the impact that iron mimics might have on iron availability and utilization in the host. On the basis of this report and others (Bernstein, 1998, 2005), gallium administration does not appear to seriously interfere with patient iron availability or utilization. In this study, there were no significant differences in serum iron concentration, total iron binding capacity, or percent transferrin saturation between GaM and control foals. In addition, the irreducibility of Ga^{3+} appears to prevent it from entering Fe^{2+} binding molecules (e.g., heme) and having an impact on normal cellular functions (Logan *et al.*, 1981). In this study, there were no differences in RBC concentrations or indices, or hemoglobin concentrations between groups.

A limitation of the study was that the small number of animals and relatively short duration of treatment restricted its

statistical power. The absence, however, of significant differences in any of the parameters between groups at 0 h and 144 h provides valuable information relative to the absence of GaM-related toxicities following short-term multiple enteral administrations in neonatal foals. This information is crucial for the development of future studies regarding the safety and effectiveness of GaM therapy in foals and in other species.

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APPENDIX 1

Clinical scoring system used for assessment of foals prior to and following 5 daily administrations of GaM (20 mg/kg) or distilled water (controls).

Variable	Score
Physical activity	
Normal - bright & alert (BAL), readily walks w/o stimulus	0
Slight depression – not BAL, walks w/o stimulus, occasionally ears down	1
Moderate depression – stands still w/o noise or action stimulus, head & ears down, nursing adequate	2
Severe depression – recumbent, slowly stands with stimulus, nursing inadequate	3
Moribund – recumbent, little or no response to stimulus	4
Fecal consistency	
Normal	0
Semi-soft, formed	1
Soft, not formed, cow paddy consistency	2
Watery, excess gas, slight abdominal discomfort	3
Projectile or constipated, excess gas, moderate to severe abdominal discomfort	4
Respiratory effort	
Normal at rest* and with exercise**	0
Normal at rest, slightly increased with exercise	1
Slight increase at rest, moderate increase with exercise	2
Moderate increase at rest, marked increase with exercise	3
Marked to severe effort at rest and with exercise	4

*Rest = foal observed from outside the stall, unprovoked; **Exercise = observer slowly pursues foal 2 to 3 times around stall with its dam.