

Serum and tissue concentrations of gallium after oral administration of gallium nitrate and gallium maltolate to neonatal calves

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OBJECTIVE

To determine serum and tissue concentrations of gallium (Ga) after oral administration of gallium nitrate (GaN) and gallium maltolate (GaM) to neonatal calves.

ANIMALS

8 healthy neonatal calves.

PROCEDURES

Calves were assigned to 1 of 2 groups (4 calves/group). Gallium (50 mg/kg) was administered as GaN or GaM (equivalent to 13.15 mg of Ga/kg for GaN and 7.85 mg of Ga/kg for GaM) by oral gavage once daily for 5 days. Blood samples were collected 0, 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hours after Ga administration on day 1; 4 and 24 hours after Ga administration on days 2, 3, and 4; and 4, 12, and 24 hours after Ga administration on day 5. On day 6, calves were euthanized and tissue samples were obtained. Serum and tissue Ga concentrations were measured by use of mass spectrometry.

RESULTS

Data were adjusted for total Ga dose, and comparisons were made between the 2 groups. Calves receiving GaM had a significantly higher dose-adjusted area under the curve and dose-adjusted maximum serum Ga concentration than did calves receiving GaN. Despite receiving less Ga per dose, calves receiving GaM had tissue Ga concentrations similar to those for calves receiving GaN.

CONCLUSIONS AND CLINICAL RELEVANCE

In this study, calves receiving GaM had significantly higher Ga absorption than did calves receiving GaN. These findings suggested that GaM might be useful as a prophylactic agent against *Mycobacterium avium* subsp *paratuberculosis* infection in neonatal calves. (*Am J Vet Res* 2016;77:151–155)

Mycobacterium avium subsp *paratuberculosis*, the causative agent of paratuberculosis (Johne disease), is a facultative intracellular bacterium that is able to survive and reproduce within monocytes and macrophages.¹ Iron is crucial for survival and replication of MAP, thereby providing a potential target for prophylactic and therapeutic strategies.² Gallium, a trivalent semimetal that shares many similarities with ferric iron and functions as an iron mimic, has antimicrobial activity against various microorganisms,^{3,4} including intracellular bacteria such as MAP.⁵ Antimicrobial activity of Ga was also reported for a study⁶

in which neonatal calves prophylactically treated with GaN before and after challenge-exposure with a live field strain of MAP had a decrease in MAP tissue colonization. However, despite a significant reduction in MAP bioburden in the treated calves, none of them were completely protected against infection.⁶ It has been suggested⁷ that GaM, a novel preparation of Ga, has higher Ga oral bioavailability and is more lipid soluble, compared with results for GaN. In a more recent study,⁸ GaM was more efficient than GaN for inhibiting MAP growth in vitro. Bioavailability of GaM has been determined for humans,⁷ foals,⁹ and adult horses,¹⁰ but to the authors' knowledge, it has not been evaluated in neonatal calves.

The objectives of the study reported here were to determine pharmacokinetics of GaN and GaM after oral administration to healthy neonatal calves and to measure tissue concentrations of Ga after oral administration to neonatal calves. We hypothesized that GaM would have superior oral absorption, when compared with oral absorption for GaN.

ABBREVIATIONS

AUC	Area under the curve
C _{max}	Maximum serum concentration
Ga	Gallium
GaM	Gallium maltolate
GaN	Gallium nitrate
K _{el}	Elimination rate constant
MAP	<i>Mycobacterium avium</i> subsp <i>paratuberculosis</i>
TP	Total protein

Materials and Methods

Animals

Eight healthy male Holstein calves (24 to 72 hours old) that weighed between 33 and 56 kg (mean, 43 kg) were assigned by use of a randomization procedure (in blocks of 2) to 1 of 2 treatment groups (4 calves/group). Calves were housed individually and fed 2 L of nonmedicated milk replacer^a (23% protein and 19% fat) twice daily via a bottle. Calves had ad libitum access to water. Before the start of the study, a blood sample was obtained from each calf and used for determination of PCV and plasma TP concentration. The study was approved by the University of Pennsylvania Institutional Animal Care and Use Committee.

Ga administration

Beginning on day 1, the calves received GaN^b or GaM^c (50 mg/kg, equivalent to 13.15 mg of Ga/kg for GaN and 7.85 mg of Ga/kg for GaM). The GaN or GaM was diluted in a small amount of milk replacer and administered orally with a dose syringe. Treatments were administered before the morning feeding; treatments were administered once daily for 5 days. Calves were observed for adverse drug reactions during the study.

Collection and processing of blood and tissue samples

Before the start of the study, an area over the left or right jugular vein of each calf was clipped and aseptically prepared. A 16-gauge, 8.3-cm catheter was inserted into the jugular vein and sutured to the skin of the neck. Prior to collection of each blood sample, a syringe that contained 2 mL of heparinized saline (0.9% NaCl) solution and was affixed to a hypodermic needle (presample syringe) was used to aspirate 10 mL of blood from the catheter. A blood sample (7 mL) was then collected with another syringe affixed to a hypodermic needle (sample syringe). The blood sample was immediately transferred into a glass blood collection tube that did not contain additives. Blood in the presample syringe was reinjected into the catheter, and the catheter was then flushed with 5 mL of heparinized saline solution. For all calves, blood samples were obtained immediately before administration of GaN or GaM (time 0) and 0.25, 0.5, 1, 2, 4, 8, 12, and 24 hours after Ga administration on day 1; 4 and 24 hours after Ga administration on days 2, 3, and 4; and 4, 12, and 24 hours after Ga administration on day 5.

Blood samples were centrifuged for 15 minutes at 1,400 X g to separate serum from the remaining blood components. Serum was divided into 2 aliquots and stored at -80°C until analysis was performed.

Calves were euthanized on day 6 by IV administration of an overdose of barbiturates. Necropsy was performed on each calf, and samples (2 g) of the liver, an ileocecal lymph node, and the ileum were collected. Tissue samples were transferred to individual sterile plastic bags and stored at -80°C until analysis was performed.

Determination of Ga concentrations

All analyses were performed at the Pennsylvania Animal Diagnostic Laboratory System of the New Bolton Center Toxicology Laboratory, School of Veterinary Medicine, University of Pennsylvania. The Ga concentration in each sample was measured by use of inductively coupled plasma mass spectrometry.^d

Frozen samples were thawed at 37°C and analyzed in batches. For tissue samples, a thoroughly mixed portion (0.4 g [wet weight]) of each sample was placed into a perfluoroalkoxy vial with 5.0 mL of 70% nitric acid and digested overnight at 70°C. Serum samples were mixed with 1.0 mL of 100% nitric acid and digested overnight at 70°C. After samples were digested, they were cooled to room temperature (22°C). Then, an internal standard (Germanium-74) was added (final concentration, 20 ng of Germanium-74/L) to 0.3 mL of digested serum or 0.2 mL of digested tissue; this mixture was diluted with deionized water (final volume, 10 mL). Diluted samples were analyzed by use of mass spectrometry. All control serum and liver samples were tested for the absence of Ga before start of the study. Method quantification limits were established at 0.01 µg/L in serum and 0.05 µg/g in liver samples on the basis of a minimum of 10 times the signal-to-noise ratio. Both Ga-69 and Ga-71 isotopes were monitored for interference, and Ga-71 was used for quantitative analysis.

A standard curve used for measuring Ga concentrations (0.00001 to 0.1 µg/L) was prepared in 2% nitric acid. The curve was found to be linear with a correlation coefficient of 0.999. A control serum sample was fortified with various concentrations of Ga (0, 0.1, 0.5, 1, 1.5, and 2 µg/L). Recovery for a duplicate set of serum samples was between 101% and 109% (relative SD, 2.9% to 4.5%). Similarly, a control liver sample was fortified with various concentrations of Ga (0, 0.05, 0.1, 0.2, 0.3, and 0.4 µg/g). Recovery for a duplicate set of liver samples was between 91% and 105% (relative SD, 1.0% to 4.9%).

Pharmacokinetic and statistical analysis

Mean body weight, PCV, and plasma TP concentration were compared between the 2 groups of calves by use of a Student *t* test. For both treatment groups, the AUC for serum concentration versus time was calculated with the trapezoidal rule by use of data for the first dose. Extrapolation from 24 hours to infinity was calculated as the concentration at 24 hours divided by the Kel. The C_{max} and time to C_{max} were determined by visual inspection of the concentration-versus-time curves for data obtained after administration of the first dose. The Kel was determined via linear regression of time versus the natural logarithm of the concentration by use of data for the last 3 times (8, 12, and 24 hours) after the first dose. The apparent absorption rate constant was determined by use of the method of residuals following extrapolation of the terminal phase of the curve to time 0 and subtraction of observed concentrations from extrapolated concentra-

tions. The AUC calculations and determination of rate constants by use of linear regression were performed with a spreadsheet program.^c The apparent absorption half-life and elimination half-life were calculated as $0.693/K_{el}$ or $0.693/K_{abs}$, respectively, where K_{abs} is the apparent absorption rate constant. Because of the difference in Ga content between GaN (26.3 weight percentage) and GaM (15.7 weight percentage), separate values for AUC and C_{max} were calculated on the basis of the amount of Ga received (AUC/Dose and C_{max} /Dose, respectively), where Dose is the dose of Ga (ie, percentage of Ga in compound) \times 50 mg/kg (equivalent to 13.15 mg of Ga/kg for GaN and

7.85 mg of Ga/kg for GaM). For tissue Ga concentrations, descriptive statistics were used and all data were reported as mean \pm SD. Values were considered significant at $P \leq 0.05$.

Results

Mean body weight before start of the study was 42 kg (range, 37 to 45 kg) for the GaN calves and 45 kg (range, 33 to 56 kg) for the GaM calves. Mean PCV before the start of the study was 28% (range, 26% to 29%) for the GaN calves and 30% (range, 26% to 38%) for the GaM calves. Mean plasma TP concentration before the start of the study was 4.3 g/dL (range, 3.5 to 5 g/dL) for the GaN calves and 4.8 g/dL (range, 4.2 to 5.6 g/dL) for the GaM calves. There was no significant difference in body weight, PCV, and plasma TP concentration between the groups, which indicated that the randomization procedure was effective.

Mean \pm SD serum concentrations of Ga were plotted versus time for both groups of calves (**Figure 1**). Mean \pm SD C_{max} for calves receiving GaN and GaM was $1,618 \pm 370$ ng/mL and $1,708 \pm 160$ ng/mL, respectively. Although both groups received doses of 50 mg/kg, calves in the GaN group received approximately 60% more Ga than did calves in the GaM group because of the difference in Ga content of each compound. Differences in the dose of Ga received were calculated as AUC/Dose and C_{max} /Dose (**Table 1**). After accounting for the difference in the amount of Ga received by each group of calves, a significant difference was found in AUC/Dose and C_{max} /Dose, whereby calves treated with GaM had significantly

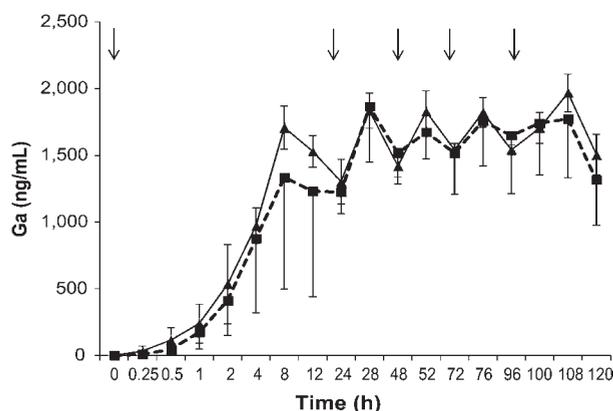


Figure 1—Mean \pm SD serum Ga concentration after oral administration (50 mg/kg) of GaN (squares and dashed line) or GaM (triangles and solid line) once daily for 5 days to neonatal calves (4 calves/treatment). The times for administration of GaN or GaM are indicated (arrows).

Table 1—Mean \pm SD values for pharmacokinetic parameters after oral administration (50 mg/kg) of GaN and GaM to neonatal calves (4 calves/treatment) once daily for 5 days.

Parameter	GaN		GaM		P value
	Mean \pm SD	Range	Mean \pm SD	Range	
AUC (ng•h/mL)	83,778 \pm 12,911	64,803–92,399	111,456 \pm 34,579	76,156–159,033	0.22
AUC/Dose (kg•h/mL)*	6.4 \pm 1.0	4.9–6.7	14.2 \pm 4.4	9.7–20.3	0.03
C_{max} (ng/mL)	1,618 \pm 370	1,229–2,078	1,708 \pm 160	1,518–1,898	0.82
C_{max} /Dose (kg/mL)*	0.12 \pm 0.03	0.09–0.16	0.24 \pm 0.06	0.19–0.33	0.01
T_{max} (h)	13.0 \pm 7.6	8.0–24.0	8.0	8.0	0.29
K_{el} (h^{-1})	0.02 \pm 0.01	0.02–0.03	0.02 \pm 0.01	0.01–0.03	0.18
$T_{1/2el}$ (h)	30.2†	23.0–46.2	39.0†	26.1–59.3	0.19
$T_{1/2abs}$ (h)	2.5†	1.7–4.2	2.7†	1.8–4.2	0.29

Values were considered significant at $P \leq 0.05$.

*Values calculated on the basis of the amount of Ga received by each group of calves. †Harmonic mean.

AUC/Dose = The AUC divided by the dose of Ga received, where Dose is the dose of Ga (ie, percentage of Ga in compound) \times 50 mg/kg. C_{max} /Dose = The C_{max} divided by the dose of Ga received. T_{max} = Time to reach C_{max} . $T_{1/2el}$ = Elimination half-life. $T_{1/2abs}$ = Apparent absorption half-life.

Table 2—Mean \pm SD Ga concentration (μ g/g) in various tissues after oral administration (50 mg/kg) of GaN or GaM to neonatal calves (4 calves/treatment) once daily for 5 days.

Tissue	GaN		GaM		P value
	Mean \pm SD	Range	Mean \pm SD	Range	
Liver	2.61 \pm 0.61	2.06–3.42	2.83 \pm 0.1	2.68–2.9	0.5
Ileocecal lymph node	1.59 \pm 0.57	1.11–2.34	1.75 \pm 0.34	1.46–2.17	0.6
Ileum	2.79 \pm 0.99	1.56–3.68	3.55 \pm 0.52	3.09–4.17	0.2

Values were considered significant at $P \leq 0.05$.

higher values for apparent absorption and Cmax than did calves treated with GaN.

Gallium was detected in all tissue samples from all treated calves. No significant difference was detected in the mean tissue Ga concentration between the treatment groups (Table 2).

Discussion

In the study reported here, Cmax values obtained after administration of GaN and GaM to neonatal calves were comparable with values obtained for neonatal foals. Mean Cmax of foals receiving 20 mg of GaM/kg via intragastric intubation was 1,079 ng/mL.⁹ Investigators of a study⁵ conducted to evaluate the in vitro activity of GaN against 10 wild strains of MAP by use of a broth culture system found that the concentration of GaN resulting in 90% growth inhibition ranged between < 200 and 743 μM (median, 440 μM) for the tested strains. On the basis of those values, serum Ga concentrations between 13,940 and 51,787 ng/mL would be expected to be therapeutic, depending on the MAP strain. In the present study, neither GaN nor GaM achieved serum concentrations equivalent to those required to suppress MAP growth in vitro. However, in vitro susceptibility results may not translate to in vivo situations because the therapeutic effectiveness of Ga against MAP is dependent on the Ga concentration in certain cells (principally macrophages, which are the target cells for MAP¹¹), rather than on the serum or raw tissue concentration alone. When administered orally, Ga becomes bound almost entirely to the ferric sites on plasma transferrin.³ Gallium is preferentially taken up by phagocytic cells at sites of inflammation and enters macrophages via transferrin-dependent and transferrin-independent mechanisms.^{12,13} Moreover, the uptake of holotransferrin (metal-saturated transferrin) is increased in macrophages infected with *Mycobacterium tuberculosis* or *M. avium*.^{14,15} Intracellular Ga concentrations were not assessed in macrophages from clinically normal calves in the study reported here.

Additionally, it is important to note that the aforementioned serum therapeutic range is extrapolated from antimicrobial susceptibility testing conducted in broth culture by use of a mycobacterial detection system in which basal iron content is higher than the iron concentrations found in vivo in serum and tissues. The difference can be illustrated by the following example. The total concentration of iron in H79 broth culture is approximately 10.3 μg/mL, compared with an iron concentration of approximately 2 μg/mL in blood. Therefore, because Ga and iron compete for acquisition by mycobacteria² and Ga-induced growth inhibition is prevented in the presence of excess iron,² it can be assumed that lower concentrations of Ga may be needed in vivo to inhibit MAP growth than those required by use of mycobacterial detection systems. Further in vitro bacterial susceptibility testing in which the basal amount of iron content in the growth media is

controlled to mimic the concentration found in vivo is needed to confirm this hypothesis.

After we accounted for the difference in the amount of Ga administered to the calves in each treatment group, GaM was found to be better absorbed than GaN. This is consistent with the literature on bioavailability of various Ga compounds, with GaM having superior bioavailability to that of other Ga salts.⁷ When dissolved in aqueous solution, Ga salts dissociate into Ga hydroxide and corresponding acids, which renders them poorly lipid soluble.⁷ However, GaM is a metal-organic coordination complex that is soluble in both water and lipids and thus can penetrate cell membranes.⁷ Similarly, although there were no significant differences in Ga tissue concentrations between groups, we can speculate that tissue absorption was better in calves receiving GaM than in those receiving GaN because calves of the GaM group received less Ga daily but tissue concentrations were similar between groups.

The time to Cmax was prolonged for both Ga formulations tested (13 and 8 hours for GaN and GaM, respectively). For this reason, Kel was determined by use of only 3 data points. The authors acknowledge that this could have affected the accuracy of Kel and AUC because Kel was used to calculate AUC.

In the study reported here, after we adjusted for the dose of Ga administered, calves receiving GaM had significantly higher Ga absorption and Cmax than did calves receiving GaN. On the basis of results of a previous study⁶ conducted by our research group, in which neonatal calves that received 20 mg of GaN/kg had a significant decrease in MAP tissue colonization but were not fully protected against infection, it may be suggested that GaM could be useful as a potential prophylactic agent against MAP infection in neonatal calves. However, additional studies involving multiple dose ranges would be necessary to determine the most effective regimen for neonatal and older calves.

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Footnotes

- a. Gehman Feed Mill Inc, Denver, Pa.
- b. Sigma-Aldrich Co, Milwaukee, Wis.
- c. Norac Pharma, Azusa, Calif.
- d. NexION, PerkinElmer, Shelton, Conn.
- e. Microsoft Excel, version 14.4.8, Microsoft Office, Microsoft Corp, Redmond, Wash.

References

1. Lambrecht RS, Collins MT. *Mycobacterium paratuberculosis*: factors which influence mycobactin-dependence. *Diagn Microbiol Infect Dis* 1992;15:239-246.
2. Olakanmi O, Britigan BE, Schlesinger LS. Gallium disrupts iron metabolism of mycobacteria residing within human macrophages. *Infect Immun* 2000;68:5619-5627.
3. Bernstein LR. Gallium therapeutic effects. In: Kretsinger RH, Uver-

- sky VN, Permyakov EA, eds. *Encyclopedia of metalloproteins*. New York: Springer-Verlag Inc, 2013;823-835.
4. Bonchi C, Imperi F, Minandri F, et al. Repurposing of gallium-based drugs for antibacterial therapy. *Biofactors* 2014;40:303-312.
 5. Fecteau ME, Fyock TL, McAdams SC, et al. Evaluation of the in vitro activity of gallium nitrate against *Mycobacterium avium* subsp. *paratuberculosis*. *Am J Vet Res* 2011;72:1243-1246.
 6. Fecteau ME, Whitlock RH, Fyock TL, et al. Antimicrobial activity of gallium nitrate against *Mycobacterium avium* subsp. *paratuberculosis* in neonatal calves. *J Vet Intern Med* 2011;25:1152-1155.
 7. Bernstein LR, Tanner T, Godfrey C, et al. Chemistry and pharmacokinetics of gallium maltolate, a compound with high oral gallium bioavailability. *Met Based Drugs* 2000;7:33-47.
 8. Fecteau ME, Aceto HW, Bernstein LR, et al. Comparison of the antimicrobial activities of gallium nitrate and gallium maltolate against *Mycobacterium avium* subsp. *paratuberculosis* in vitro. *Vet J* 2014;202:195-197.
 9. Martens RJ, Mealey K, Cohen ND, et al. Pharmacokinetics of gallium maltolate after intragastric administration in neonatal foals. *Am J Vet Res* 2007;68:1041-1044.
 10. Arnold C, Chaffin MK, Cohen N, et al. Pharmacokinetics of gallium maltolate after intragastric administration in adult horses. *Am J Vet Res* 2010;71:1371-1376.
 11. Sweeney RW. Pathogenesis of paratuberculosis. *Vet Clin North Am Food Anim Pract* 2011;27:537-546.
 12. Tsan MF. Mechanism of gallium-67 accumulation in inflammatory lesions. *J Nucl Med* 1985;26:88-92.
 13. Chitambar CR, Zivkovi Z. Uptake of gallium-67 by human leukemic cells: demonstration of transferrin receptor-dependent and transferrin-independent mechanisms. *Cancer Res* 1987;47:3929-3934.
 14. Halaas O, Steigedal M, Haug M, et al. Intracellular *Mycobacterium avium* intersect transferrin in the Rab11(+) recycling endocytic pathway and avoid lipocalin 2 trafficking to the lysosomal pathway. *J Infect Dis* 2010;201:783-792.
 15. Boradia VM, Malhotra H, Thakkar JS, et al. *Mycobacterium tuberculosis* acquires iron by cell-surface sequestration and internalization of human holo-transferrin. *Nat Commun* [serial online].