Pharmacokinetics of gallium maltolate after intragastric administration in neonatal foals

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Objective—To determine the pharmacokinetics of gallium maltolate (GaM) after intragastric administration in healthy foals.

Animals—6 healthy neonatal foals.

Procedures—Each foal received GaM (20 mg/kg) by intragastric administration. Blood samples were obtained before (time 0) and at 0.25, 0.5, 1, 2, 4, 8, 12, 24, 36, and 48 hours after GaM administration for determination of serum gallium concentrations by use of inductively coupled plasma mass spectroscopy.

Results—Mean ± SD pharmacokinetic variables were as follows: peak serum gallium concentration, 1,079 ± 311 ng/mL; time to peak serum concentration, 4.3 ± 2.0 hours; area under the serum concentration versus time curve, 40,215 ± 8,420 ng/mL/h; mean residence time, 39.5 ± 17.2 hours; area under the moment curve, 1,636,554 ± 931,458 ng([h]^2/mL); and terminal half-life, 26.6 ± 11.6 hours. The mean serum concentration of gallium at 12 hours was 756 ± 195 ng/mL.

Conclusions and Clinical Relevance—Gallium maltolate administered via nasogastric tube at a dose of 20 mg/kg to neonatal foals resulted in gallium serum concentrations considered sufficient to suppress growth or kill *Rhodococcus equi* in macrophages and other infected tissues. (*Am J Vet Res* 2007;68:1041–1044)

Rhodococcus equi is a facultative intracellular bacterium that is able to survive and reproduce within macrophages[1] causing severe, potentially fatal, bronchopneumonia in foals and immunocompromised people.[2,3] Most foals likely become infected within the first few days of life,[4] when they may have immature or ineffective innate immune responses.[5-8] Thus, strategies designed to prevent or ameliorate these early infections may provide effective disease control.

Ferric iron (Fe3+) sequestration by host proteins, primarily transferrin, but also lactoferrin and ferritin, is an innate defense mechanism that limits availability of iron to most pathogenic microbes. *Rhodococcus equi*, however, which is dependent on adequate ferric iron for survival, can acquire and use transferrin and lactoferrin-bound iron, thereby circumventing this defense mechanism.[9] Strategies designed to exploit iron dependency of various pathogenic organisms have proven effective in the management of a variety of bacterial diseases.[10-13]

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Abbreviations

GaM	Gallium maltolate
AUC	Area under the serum concentration versus time curve
Cmax	Maximum serum concentration
Tpeak	Time to reach maximum serum concentration
MRT	Mean residence time

Gallium, 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.[10,13-15] Gallium, particularly when administered orally, readily binds to plasma transferrin and lactoferrin[14-16] and subsequently concentrates at sites of infection and inflammation and in macrophages,[17-19] the target cell of *R equi*. As an iron mimic, gallium is incorporated into crucial iron-dependent DNA-synthesis enzyme systems of certain bacteria, causing inactivation of those enzymes and bacterial death.[14] Gallium has bacteriostatic and bactericidal activity against *R equi* in vitro and in vivo.[9,10]

Gallium maltolate, a coordination complex of gallium and maltol, provides high gallium bioavailability following oral administration in humans and a variety of other species and has not been associated with substantial toxic effects or gastrointestinal irritation.[16] Prophylactic intragastric administration of GaM to experimentally infected mice reduced their *R equi* tissue burdens.[10] The study reported here was designed to determine the pharmacokinetics of intragastrically administered GaM in neonatal foals.

Materials and Methods

Animals and procedures—Six 1- to 2-day-old Quarter Horse foals of either sex (3 male and 3 female) weighing 38 to 52 kg (mean, 48.5 kg) were studied. Each foal received adequate transfer of maternal antibodies (serum IgG ≥ 800 mg/dL) as determined by a commercial assay.[a] Foals were deemed healthy on the basis of findings on physical examination, CBC, and serum biochemical profile. Each foal was housed in a box stall with its dam and allowed to nurse ad libitum. Foals received a distilled water solution of GaM (10 mg/mL) via nasogastric tube at a dose of 20 mg/kg. Foals were observed for adverse reactions during the study. Blood samples were collected for measurement of serum gallium concentration prior to (0 hours) and at 0.25, 0.5, 1, 2, 4, 8, 12, 24, 36, and 48 hours after GaM administration. Serum was harvested and stored at –80°C, and all samples were assayed for gallium at the same time. The study was approved by the Texas A&M University Institutional Animal Care and Use Committee.

Determination of gallium concentrations—Gallium concentrations were measured by inductively coupled plasma mass spectroscopy. Serum samples were thawed at 37°C and diluted with 1% ultrapure HNO_3 in deionized water,[b] in preparation for gallium analysis by inductively coupled plasma-mass spectroscopy[c] with the isotopes gallium Ga 71 and rhodium Rh 103 (as internal standards). Weight linear calibration was performed with a blank and 4 external standards (0.2, 2.0, 20, and 200 ng/mL). Data were acquired in peak hopping mode by use of the autolens feature and 3 replicate reads per determination. Calibration and baseline determinations were performed before and after the analytic runs. The inductively coupled plasma-mass spectroscopy detection limit for gallium in serum was 0.5 ng/mL, and method blanks averaged 0.6 ng/mL, well below the 1.5 ng/mL limit of quantification. Analytic precision and accuracy were acceptable. The relative percent difference (range divided by the mean value) of 9 duplicate pairs averaged 2%, whereas recovery of gallium added to 8 blanks (spiked blanks per laboratory control samples) and 8 samples (matrix spikes) averaged 107% and 100%, respectively. Instrumental response was linear over a calibration range (0 to 20 ng/mL), with a correlation coefficient (R^2) of 0.9999 and a coefficient of variation of 1.4%.

Pharmacokinetic analysis—The AUC (calculated by the trapezoidal method), Cmax, Tpeak, and MRT of gallium were determined by use of a nonlinear curve-fitting program.[d] Descriptive statistics were determined with commercially available software,[e] and all data were reported as mean ± SD.

Results

Serum concentrations of gallium were plotted versus time for each foal (**Figure 1**). For each foal, quantifiable gallium concentrations were detectable by the first time point (0.25 hours). Mean pharmacokinetic variables calculated for gallium included Cmax, Tpeak, AUC, MRT, area under the moment curve, and the terminal elimination half-life (**Table 1**). Mean Cmax for the foals was 1,079 ± 311 ng/mL, and 5 of 6 foals had a Cmax > 700 ng/mL. Mean Tpeak was 4.3 ± 2.0 hours, indicating rapid GaM absorption following intragastric administration. The mean serum concentration of gallium at 12 hours was 756 ± 195 ng/mL, with 5 of 6 foals having serum concentrations exceeding 700 ng/mL at 12 hours. Mean AUC, which is a measure of total exposure, was 40,215 ± 8,420 ng/mL/h, and MRT was 39.5 ± 17.2 hours. The area under the moment curve was 1,636,554 ± 931,458 ng([h]²/mL), and the terminal half-life was 26.6 ± 11.6 hours.

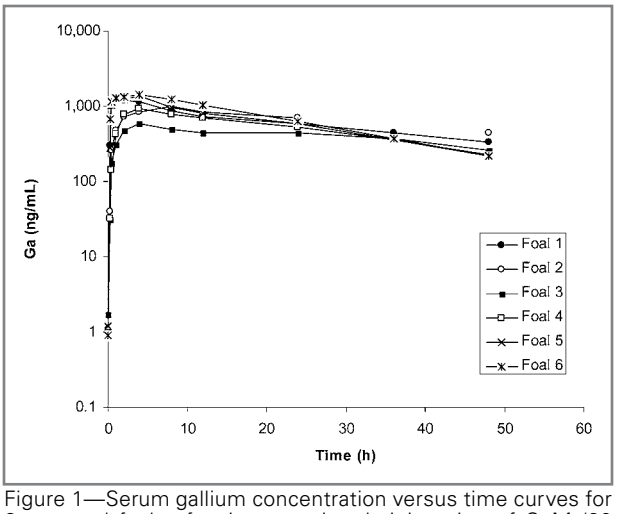

Figure 1—Serum gallium concentration versus time curves for 6 neonatal foals after intragastric administration of GaM (20 mg/kg).

Table 1—Pharmacokinetic values of GaM (20 mg/kg) after intragastric administration in 6 neonatal foals.

Variables	Mean ± SD	Range
AUC (ng/mL/h)	40,214 ± 8,420	32,205–56,108
MRT (h)	39.5 ± 17.2	23.9–66.4
Cmax (ng/mL)	1,079 ± 311	588–1,410
Tpeak (h)	4.3 ± 2.0	2.0–8.0
$t_{1/2\lambda}$ (h)	26.6 ± 11.6	16.2–45.1
AUMC ng([h]²/mL)	1,636,554 ± 931,458	818,778–3,008,467

$t_{1/2\lambda}$ = Terminal elimination half-life. AUMC = Area under the moment curve.

Discussion

For the purposes of our study, a serum gallium concentration of 700 ng/mL was considered therapeutic. The therapeutic effectiveness of gallium against *R equi* is dependent on its concentration in infected tissues, principally macrophages, which are the target cell for *R equi*,[1] rather than serum concentrations alone. Gallium, which exists in plasma predominantly bound to the ferric (Fe^{3+}) sites on transferrin, is preferentially taken up by phagocytic cells at sites of inflammation, avidly accumulates at sites of infection and granulomatous lesions, and enters macrophages via both transferrin-dependent and transferrin-independent mechanisms.[17-19] The designated therapeutic serum concentration (ie, 700 ng/mL) was based on a couple of factors. First, in

murine macrophage-like (J774A.1) cells experimentally infected with virulent *R equi*, a 10µM concentration of GaM (elemental gallium, 697 ng/mL) in tissue culture media significantly reduced intracellular concentrations of *R equi*, compared with those in GaM-free media.[20] Second, in mice treated prophylactically with GaM and experimentally infected with virulent *R equi*, it was deduced that a serum gallium concentration of 700 ng/mL reduces *R equi* tissue burdens by approximately 90%.[10] Because gallium preferentially accumulates in activated phagocytic cells (eg, infection and inflammation),[17,18] intracellular gallium concentrations were not assessed in macrophages from clinically normal foals in our study.

When gallium salts, such as chloride or nitrate, are dissolved in aqueous solution, they dissociate into gallium hydroxide species and the corresponding acids (eg, hydrochloric or nitric), leaving the resulting solution highly acidic and unsuitable for oral or parenteral administration.[14] In addition, precipitation of the gallium as insoluble hydrated hydroxides in the gastrointestinal tract contributes to the low bioavailability of gallium from orally administered salts. A citrate-chelated gallium nitrate solution for injection[f] is approved in the United States for the treatment of human cancer-related hypercalcemia. This formulation is administered as a continuous IV infusion for 5 days at 5 mg/kg/d to avoid toxic effects on the renal system that can occur with bolus IV administration.[21] Toxic effects of GaM on the renal system has not been associated with GaM; presumably, this is because nearly all the gallium from GaM following oral administration becomes protein bound in the blood, whereas that from gallium nitrate following IV administration is largely present as ionic species that rapidly concentrate in and are eliminated by the kidneys.[16]

Gallium maltolate, developed by an author of our study (LRB), has gallium bioavailability of ≥ 25% to 57% after oral administration in healthy humans, with roughly linear absorption and elimination kinetics following single doses of 100 to 500 mg and a mean Cmax of 569 ng/mL at the 500-mg dose.[16] The actual oral bioavailability of gallium in foals was not determined in our study because this calculation requires AUC data obtained following IV administration, which was not performed because a suitable IV formulation of GaM was not available. However, bioavailability appears to be sufficient to achieve gallium concentrations considered therapeutic.

The disposition of GaM in neonatal foals would be expected to differ somewhat from older foals. Changes in the physiologic processes that affect drug absorption, distribution, and metabolism and excretion occur as the foal matures. For example, absorption of an orally administered drug depends on gastric emptying time, which may be different in an older foal. Similarly, differences in body fluid compartments and plasma proteins can alter drug distribution. Finally, maturation of liver and kidney function can substantially alter drug metabolism and excretion.

Estimated pharmacokinetic parameters for GaM versus gallium nitrate after intraduodenal administration in dogs at a dose of 1.5 mg gallium/kg were as follows, respectively: Cmax, 2,200 ng/mL versus 310 ng/mL; Tpeak, 0.5 hours versus 3.1 hours; and AUC, 37,000 ng/mL/h versus 5,600 ng/mL/h.[16] In mice, serum concentrations of gallium were measured after 10 days of GaM treatment (10 mg/kg or 50 mg/kg, oral gavage, q 24 h); at 2 hours after oral administration on treatment day 10, mean serum concentrations of gallium were 110.5 ng/mL and 559.3 ng/mL for GaM doses of 10 mg/kg and 50 mg/kg, respectively.[10] For GaM administration on a milligram per kilogram basis, serum gallium concentrations were substantially less in mice than in foals. Whether this represents greater bioavailability in foals, compared with mice, is unknown.

To our knowledge, our study provides the first data on GaM administration to horses and gallium disposition in horses. At a dose of 20 mg/kg, GaM appears to be adequately bioavailable in foals and might therefore be useful for prophylaxis or treatment against *R equi*. Furthermore, GaM (20 mg/kg) is rapidly absorbed following intragastric administration, achieving gallium serum concentrations and ostensibly tissue concentrations that, on the basis of findings on murine and macrophage cell lines, should be adequate to suppress growth and kill intracellular *R equi* for at least 24 hours. However, additional studies involving multiple doses of GaM are necessary to determine appropriate dose regimens of GaM for neonatal and older foals. Prophylactic GaM treatment during the first weeks of the life of a foal may afford adequate protection against early infection with *R equi,* thereby providing additional time for maturation of requisite innate and adaptive immune functions. This could substantially reduce the incidence of disease on *R equi* endemic farms. In addition, GaM used alone or in conjunction with standard antimicrobial protocols may be valuable for the treatment of established *R equi* infections.

a. Snap Test, IDEXX Laboratories, Westbrook, Me.
b. Seastar Baseline, Seastar Chemicals Inc, Sidney, BC, Canada.
c. Model DRC 2, Perkin Elmer, Foster City, Calif.
d. PK Analyst, MicroMath, Salt Lake City, Utah.
e. Microsoft Excel, Microsoft Corp, Redmond, Wash.
f. Ganite, Genta Inc, Berkeley Heights, NJ.

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