Gamithromycin is a novel azalide antimicrobial that has recently been developed for the treatment and prevention of bovine respiratory disease caused by Mannheimia haemolytica, Pasteurella multocida, and Histophilus somni. Azalides are similar to macrolides in that both inhibit bacterial protein synthesis by binding the 50S subunit of the prokaryotic ribosomes. However, gamithromycin differs from macrolides in its structural composition by having a 15-membered semisynthetic lactone ring with a uniquely positioned alkylated nitrogen atom at the 7a position. Macrolide antimicrobials are generally bacteriostatic. However, gamithromycin is bactericidal against M. haemolytica, P. multocida, and H. somni, with minimal bactericidal concentrations only 1 dilution higher than the respective MIC.1

**Objective**—To determine the disposition of gamithromycin in plasma, pulmonary epithelial lining fluid (PELF), bronchoalveolar lavage (BAL) cells, and lung tissue homogenate in cattle.

**Animals**—33 healthy Angus calves approximately 7 to 8 months of age.

**Procedures**—Calves were randomly assigned to 1 of 11 groups consisting of 3 calves each, which differed with respect to sample collection times. In 10 groups, 1 dose of gamithromycin (6 mg/kg) was administered SC in the neck of each calf (0 hours). The remaining 3 calves were not treated. Gamithromycin concentrations in plasma, PELF, lung tissue homogenate, and BAL cells (matrix) were measured at various points by means of high-performance liquid chromatography with tandem mass spectrometry.

**Results**—Time to maximum gamithromycin concentration was achieved at 1 hour for plasma, 12 hours for lung tissue, and 24 hours for PELF and BAL cells. Maximum gamithromycin concentration was 27.8 µg/g, 17.8 µg/mL, 4.61 µg/mL, and 0.433 µg/mL in lung tissue, BAL cells, PELF, and plasma, respectively. Terminal half-life was longer in BAL cells (125.0 hours) than in lung tissue (93.0 hours), plasma (62.0 hours), and PELF (50.6 hours). The ratio of matrix to plasma concentrations ranged between 4.7 and 127 for PELF, 16 and 650 for lung tissue, and 3.2 and 2,135 for BAL cells.

**Conclusions and Clinical Relevance**—Gamithromycin was rapidly absorbed after SC administration. Potentially therapeutic concentrations were achieved in PELF, BAL cells, and lung tissue within 30 minutes after administration and persisted for 7 (PELF) to > 15 (BAL cells and lung tissue) days after administration of a single dose. (Am J Vet Res 2011;72:326–330)

Gamithromycin is absorbed rapidly and fully following SC administration in cattle.1 The high apparent volume of distribution (mean ± SD, 24.9 ± 2.99 L/kg), long half-life (44.9 ± 4.67 hours), and low protein binding (26.0 ± 0.60%) of gamithromycin are desirable pharmacokinetic parameters for an antimicrobial used for single-dose treatment of bovine respiratory disease.1 However, optimal dosing of an antimicrobial is dependent not only on the pharmacokinetics but also on the pharmacodynamics of the drug. The pharmacodynamic properties of a drug describe the relationship between drug concentration and antimicrobial activity. Recent data indicate that traditional pharmacodynamic

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**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>AUC</td>
<td>Area under the concentration-time curve</td>
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<tr>
<td>AUMC</td>
<td>Area under the first moment of the concentration-time curve</td>
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<tr>
<td>BAL</td>
<td>Bronchoalveolar lavage</td>
</tr>
<tr>
<td>HPLC</td>
<td>High-performance liquid chromatography</td>
</tr>
<tr>
<td>K</td>
<td>Elimination rate constant</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimal inhibitory concentration</td>
</tr>
<tr>
<td>MIC₉₀</td>
<td>Minimal concentration that inhibits at least 90% of isolates</td>
</tr>
<tr>
<td>PELF</td>
<td>Pulmonary epithelial lining fluid</td>
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</table>

Gamithromycin is absorbed rapidly and fully following SC administration in cattle.1 The high apparent volume of distribution (mean ± SD, 24.9 ± 2.99 L/kg), long half-life (44.9 ± 4.67 hours), and low protein binding (26.0 ± 0.60%) of gamithromycin are desirable pharmacokinetic parameters for an antimicrobial used for single-dose treatment of bovine respiratory disease.1 However, optimal dosing of an antimicrobial is dependent not only on the pharmacokinetics but also on the pharmacodynamics of the drug. The pharmacodynamic properties of a drug describe the relationship between drug concentration and antimicrobial activity. Recent data indicate that traditional pharmacodynamic
parameters based on plasma concentrations of macrolides and azalides do not apply to the treatment of pulmonary infections because these drugs concentrate preferentially in the lungs. Plasma concentrations of macrolides or azalides such as gamithromycin, tilmicosin, and tulathromycin in cattle are, in general, considerably lower than their MICs for the pathogens for which they have been approved. Nonetheless, studies have revealed the efficacy of these drugs in the treatment of bovine respiratory disease, indicating that the amount of time drug concentrations at the site of infection remain higher than the MIC of the pathogen provides more clinically relevant information than does simple reliance on plasma concentrations.

The purpose of the study reported here was to determine the disposition of gamithromycin in plasma, PELF, BAL cells, and lung tissue of cattle following administration of 1 SC dose. It was hypothesized that gamithromycin would concentrate in PELF, BAL cells, and lung tissue.

**Materials and Methods**

**Animals**—Thirty-three healthy Angus calves (18 males and 15 females) between approximately 7 and 8 months of age were used in the study. Calves had not received any treatment for at least 21 days prior to inclusion. They were acclimated to the study facility for 7 days. The study protocol was approved by Merial’s Animal Care and Use Committee.

**Experimental design**—Calves were randomly assigned to 11 groups consisting of 3 animals each based on sample collection times. Three calves were not treated and were used as a negative control group. The remaining 30 calves were injected SC in the neck (0 hours) with gamithromycin in the proposed market formulation at a dose of 6 mg/kg. No more than 10 mL was administered in any 1 injection site. Blood samples for plasma separation were collected from a jugular vein immediately prior to drug administration (n = 30 calves; 0 hours), as well as 0.5 (30), 1 (27), 2 (27), 4 (24), 6 (21), 8 (21), and 12 (18) hours and 1 (15), 2 (12), 3 (12), 4 (9), 5 (9), 6 (9), 7 (6), 8 (6), 10 (6), and 15 (3) days after drug administration. Plasma was stored at −70°C until assayed for gamithromycin and urea concentrations.

Immediately after blood collection, a group of 3 calves was euthanized by penetrating captive bolt stunning followed by exsanguination at each of the following points: 0, 0.5, 2, 4, 8, and 12 hours and 1, 3, 7, 10, and 15 days after drug administration. The lungs were immediately removed from each calf for BAL fluid and lung tissue collection.

**BAL and lung tissue collection**—Bronchoalveolar lavage fluid was collected from harvested lungs after euthanasia of calves. An endoscope (10 mm in diameter) was passed through the trachea and advanced until wedged into a bronchus. The lavage solution consisted of 4 aliquots of 60 mL of physiologic saline (0.9% NaCl) solution infused and aspirated immediately through the endoscope. For each calf, the procedure was conducted for each lung and the mean value of gamithromycin concentration was used for data analysis. Nucleated cells were counted in BAL fluid by use of an automated cell counter. Bronchoalveolar fluid was centrifuged at 200 × g for 10 minutes, and the sedimented BAL cells were washed, resuspended in PBS solution, mixed with the aid of a vortex device, and frozen at −70°C until assayed. Supernatant BAL fluid was also frozen at −70°C until assayed.

Before the assay, the cell pellet samples were thawed, mixed vigorously with the aid of a vortex device, and sonicated to ensure complete cell lysis. The resulting suspension was centrifuged at 500 × g for 10 minutes, and the supernatant was used to determine the intracellular concentrations of gamithromycin.

In addition, approximately 2 g of tissue was collected from each lung and frozen at −70°C until assayed. Before the assay, the lung tissue was ground cryogenically in a tissue grinder and thoroughly mixed.

**Analysis of gamithromycin concentrations**—For each matrix, standard curve and quality control samples were fortified with serial concentrations of working standard solutions. Plasma and lung tissue samples were analyzed through use of validated liquid chromatography–mass spectrometry and liquid chromatography–tandem mass spectrometry methods as described elsewhere. A similar assay method was developed and validated for analysis of BAL fluid and BAL cell samples. Deuterated gamithromycin was used as an internal standard for all assays. Briefly, samples were mixed with 0.1M potassium monobasic phosphate buffer followed by centrifugation at approximately 13,000 × g for 5 minutes. The resulting supernatant was loaded into a preconditioned 96-well solid-phase extraction plate for plasma and BAL fluid and cell samples or 150 mg of solid phase extraction cartridges for lung tissue samples. The eluted samples were analyzed by means of tandem mass spectrometry with electrospray ionization in positive ion mode.

Standard curves for plasma ranged from 0.002 to 1.0 µg/mL. The lower limit of quantification for gamithromycin in plasma was 0.002 µg/mL. Mean recoveries of gamithromycin from spiked plasma control samples ranged from 84% to 118%. Standard curves for BAL cell and BAL fluid ranged from 0.010 µg/mL to 3.0 µg/mL and 0.0025 µg/mL to 1.0 µg/mL, respectively. The limit of quantification was 0.010 µg/mL for BAL cell suspensions and 0.0005 µg/mL for BAL fluid. Mean recoveries of gamithromycin from spiked BAL cell and fluid quality control samples ranged from 94% to 107% and 94% to 118%, respectively. Standard curves for lung tissue concentrations ranged from 0.100 to 10.0 µg/mL. The lower limit of quantification for gamithromycin determination in lung tissue was 0.100 µg/g. Mean recoveries of gamithromycin from spiked lung tissue control samples ranged from 90% to 112%. Regardless of the matrix, the coefficient of determination was always ≥0.99.

**Calculation of gamithromycin concentrations in PELF and BAL cells**—Pulmonary distribution of gamithromycin was determined as reported elsewhere. Estimation of the volume of PELF was determined by means of the urea dilution method. Urea nitrogen concentrations in plasma (Urea<sub>plasma</sub>) and in BAL fluid (Urea<sub>BAL</sub>) were determined by use of a commercial
kit, and the absorbance values were measured by use of a spectrophotometer. The volume of PELF \((V_{\text{PELF}})\) in BAL fluid was derived from the following equation: 

\[ V_{\text{PELF}} = V_{\text{BAL}} \times \left( \frac{\text{Urea}_{\text{BAL}}}{\text{Urea}_{\text{PLASMA}}} \right) \]

in which \(V_{\text{BAL}}\) is the volume of recovered BAL fluid. The concentration of gamithromycin PELF \((\text{GAM}_{\text{PELF}})\) was derived from the following relationship:

\[ \text{GAM}_{\text{PELF}} = \text{GAM}_{\text{BAL}} \times \left( \frac{V_{\text{PELF}}}{V_{\text{PELF}}} \right) \]

in which \(\text{GAM}_{\text{PELF}}\) is the measured concentration of gamithromycin in BAL fluid.

The concentration of gamithromycin in BAL cells \((\text{GAM}_{\text{CELL}})\) was calculated on the basis of the following relationship:

\[ \text{GAM}_{\text{CELL}} = \left( \frac{\text{GAM}_{\text{PELF}}}{V_{\text{CELL}}} \right) \]

in which \(\text{GAM}_{\text{PELF}}\) is the concentration of antimicrobial in the cell pellet supernatant and \(V_{\text{CELL}}\) is the mean volume of cattle BAL cells. A volume of 1.28 \(\mu\text{l} \times 10^7\) BAL cells, derived in another bovine study, was used for the \(V_{\text{CELL}}\) value.

**Pharmacokinetic analysis**—Plasma, lung tissue, PELF, and BAL cell mean gamithromycin concentration versus time data were analyzed on the basis of noncompartmental pharmacokinetics with the aid of computer software. The \(K_t\) was determined by linear regression of the terminal phase of the logarithmic mean concentration versus time curve by use of a minimum of 3 data points. Terminal half-life was calculated as the natural logarithm of 2 divided by \(K_t\). The AUC and AUMC were calculated on the basis of the trapezoidal rule, with extrapolation to infinity by use of \(C_{\text{min}}/K_{\text{el}}\), in which \(C_{\text{min}}\) was the final measurable gamithromycin concentration. Mean residence time was calculated as AUMC/AUC.

**Statistical analysis**—Normality of data distributions and equality of variances were assessed by use of the Kolmogorov-Smirnov and Levene tests, respectively, with the aid of statistical software. Two-way ANOVA for repeated measures was used to evaluate the effect of the matrix (PELF, BAL cells, and lung tissue), time, and the interaction between matrix and time on the ratio of gamithromycin concentration in the matrix to that in plasma. Data for variables that did not meet the assumptions of the ANOVA were rank-transformed prior to analysis. Multiple pairwise comparisons were performed by use of the Holm-Sidak test. Differences were considered significant at a value of \(P < 0.05\).

**Results**

Time to maximum gamithromycin concentration was achieved 1 hour after gamithromycin administration for plasma, 12 hours afterward for lung tissue, and 24 hours afterward for PELF and BAL cells (Table 1). Maximum gamithromycin concentration was 27.8 \(\mu\text{g/mL}\), 17.8 \(\mu\text{g/mL}\), 4.61 \(\mu\text{g/mL}\), and 0.433 \(\mu\text{g/mL}\) for lung tissue, BAL cells, PELF, and plasma, respectively. Terminal half-life was longer in BAL cells (125.0 hours) followed by lung tissue (93.0 hours), plasma (62.0 hours), and PELF (50.6 hours; Figure 1).

Gamithromycin concentrated rapidly in PELF, BAL cells, and lung tissue with the matrix-to-plasma concentration ratio ranging between 3.2 and 16 as early as 0.5 hours after administration (Table 2). The matrix-to-plasma concentration ratios ranged between 4.7 and 127 for PELF, 16 and 650 for lung tissue, and 3.2 and 17.8 for BAL cells (Table 1).

**Table 1**—Plasma, PELF, BAL cell, and lung tissue pharmacokinetic variables for gamithromycin (6 mg/kg) after SC administration in 30 healthy Angus calves.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Plasma</th>
<th>PELF</th>
<th>BAL cells</th>
<th>Lung</th>
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<tbody>
<tr>
<td>(K_t) (h(^{-1}))</td>
<td>0.0112</td>
<td>0.0129</td>
<td>0.0137</td>
<td>0.0155</td>
</tr>
<tr>
<td>(T_{\text{peak}}) (h)</td>
<td>62.0</td>
<td>50.6</td>
<td>125.0</td>
<td>93.0</td>
</tr>
<tr>
<td>(C_{\text{max}}) ((\mu\text{g/mL}) or (\mu\text{g/g}))</td>
<td>4.61</td>
<td>4.61</td>
<td>17.8</td>
<td>27.8</td>
</tr>
<tr>
<td>(\text{AUC}_{\text{PELF},\text{BAL}}) ((\mu\text{g•h/mL}) or (\mu\text{g•h/g}))</td>
<td>7.82</td>
<td>334</td>
<td>3,062</td>
<td>2,154</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Data are reported to 3 significant figures. AUC&lt;sub&gt;PELF&lt;/sub&gt; = AUC extrapolated to infinity, AUC&lt;sub&gt;PELF&lt;/sub&gt; = Percentage of AUC&lt;sub&gt;PELF&lt;/sub&gt; that was extrapolated beyond the last quantifiable point. AUC&lt;sub&gt;PELF&lt;/sub&gt; = AUC from time 0 to the last quantifiable time point (t&lt;sub&gt;1&lt;/sub&gt;). AUC&lt;sub&gt;PELF&lt;/sub&gt; = AUMC extrapolated to infinity, AUMC&lt;sub&gt;PELF&lt;/sub&gt; = AUMC from time 0 to the last quantifiable time point (t&lt;sub&gt;1&lt;/sub&gt;). C&lt;sub&gt;max&lt;/sub&gt; = Maximum concentration, MRT = Mean residence time, (t_{1/2}) = Terminal half-life, (T_{\text{peak}}) = Time to peak concentration.</td>
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</table>
There was a significant effect of the type of matrix ($P < 0.001$) and time ($P < 0.001$), as well as a significant ($P < 0.001$) interaction between matrix and time, on the matrix-to-plasma concentration ratios. The lung tissue-to-plasma gamithromycin concentration ratio was significantly higher than the PELF-to-plasma and BAL-to-plasma values for 8 hours after drug administration. Between 168 and 360 hours after administration, BAL cell-to-plasma concentration ratio was highest, followed by lung tissue-to-plasma and PELF-to-plasma values. The PELF-to-plasma concentration ratio was significantly lower than the BAL cell-to-plasma and lung tissue-to-plasma values at most time points.

### Discussion

The present study revealed that gamithromycin at 6 mg/kg was rapidly absorbed into the systemic circulation after SC administration and rapidly reached concentrations exceeding the MIC₉₀ for several common respiratory pathogens in PELF, BAL, and lung tissue of calves. Plasma concentrations of macrolides or azalides such as gamithromycin, tilmicosin, and tulathromycin in cattle are in general considerably less than the MICs of the pathogens against which these drugs are effective. It is therefore accepted that plasma concentrations of these drugs are poor predictors of in vivo efficacy against respiratory pathogens and that drug concentrations at the site of infection provide more clinically relevant information. Although drug concentration in plasma is clearly a driving force for penetration to the site of infection, the actual drug concentration-time profile at a peripheral body site may be quite different from that of plasma. The rate and extent of penetration of a drug into most sites outside the vascular space are also determined by the drug's molecular charge and size, lipid solubility, extent of plasma protein binding, and blood flow at the site of infection.

In other tissues such as the bronchial epithelium, antimicrobial diffusion to the site of infection (bronchial secretions and PELF) is further restricted by tight junctions between cells.

For pulmonary infections caused by extracellular bacteria such as *M haemolytica* and *P multocida*, concentrations of antimicrobials in the extracellular or interstitial space within the lungs would provide additional relevant information. Whereas it is common practice to measure drug concentration in tissue homogenates, the homogenization procedure disrupts cell membranes and yields a suspension containing both intracellular and extracellular fluid and particles. This typically results in considerable overestimation of antimicrobial concentrations in the extracellular environment.

Measurement of drug concentration in PELF collected via BAL is the most widely used method to estimate antimicrobial concentrations at the site of infection for antimicrobials intended to treat lower respiratory tract infections caused by extracellular bacterial pathogens in people. Antimicrobial concentrations in PELF must be interpreted with a complete understanding of the limitations of the methods used. The present study, like most similar studies in people, involved the urea dilution method to estimate the volume of PELF. Estimation of PELF volume by use of the urea dilution method may result in a false increase in BAL fluid urea concentration by diffusion of urea from the interstitium and blood when BAL fluid dwell time is prolonged. Prolonged BAL fluid dwell time was minimized in our study by use of rapid infusion of 100 mL of saline solution followed by immediate aspiration. Overestimation of urea concentrations in BAL fluid would falsely increase the volume of PELF, which would in turn result in an underestimation of drug concentrations in PELF.

Another possible confounding factor in interpreting PELF concentrations of antimicrobials measured via BAL is that the high ratios of PELF to plasma concentrations achieved with macrolides and azalides considerably exceed those that would be predicted solely on the basis of their high lipophilicity and good penetration across the alveolar epithelium. This disparity led to the hypothesis that contamination with intracellular antimicrobials may result from cell lysis during the process of BAL. Macrolides are potent weak bases that become ion-trapped within acidic intracellular compartments such as lysosomes and phagosomes. A beneficial consequence of macrolide accumulation within cells is an increase in activity against intracellular pathogens. In addition, phagocytes have been found to act as a vehicle for the delivery of macrolides to the site of infection. Therefore, an alternative hypothesis might be that the higher than predicted PELF concentrations measured are the results of drug delivery to the respiratory tract in vivo rather than an artifact of cell lysis as a result of the BAL procedure.

Despite these methodological concerns, measurement of drug concentrations in PELF for infections caused by extracellular pathogens is believed to better reflect clinical efficacy in pneumonia. In addition, high concentrations of macrolides and azalides in PELF have long been proposed as a key factor in their efficacy against respiratory pathogens in humans. The preferential activity of macrolides against extracellular pathogens in the lungs has been determined conclusively in mice infected with *Streptococcus pneumoniae* isolates with efflux-mediated macrolide resistance. In that study, consistent bactericidal activity was
detected in a lung infection model, whereas no drug effect was evident in a thigh infection model. These differences in bacterial activity between sites are explained by the much higher drug concentrations reached in PELF than in plasma. The results of the aforementioned study suggest that drug concentrations in PELF are a better predictor of outcome, than are plasma concentrations, for respiratory tract infections.

In the present study, gamithromycin concentrations in PELF, BAL cells, and lung tissue were higher than concurrent plasma concentrations as early as 0.5 hours after gamithromycin administration, and peak concentrations at these sites were achieved within 12 (lung tissue) to 24 (PELF and BAL cells) hours. Gamithromycin concentrations in PELF, lung tissue, and BAL cells were 4.7 to 127, 16 to 650, and 3.2 to 2,135 times as high as concurrent plasma concentrations, respectively. The ability of gamithromycin to concentrate at the site of infection coupled with slow elimination of drug from these sites results in sustained drug concentrations for days following a single injection.

Gamithromycin has MIC₉₀ values of 0.5, 1.0, and 1.0 μg/mL for M haemolytica, P multocida, and H somni isolated from infected cattle, respectively. These MICs are in general considerably higher than the plasma gamithromycin concentrations obtained in our study. However, a SC dose of gamithromycin at 6 mg/kg resulted in concentrations higher than the MIC₉₀ for these pathogens for 3 to 7 days in PELF and for >15 days in BAL cells and lung tissue. Gamithromycin also has an extended postantimicrobial effect against bovine respiratory pathogens, which might also contribute to the extended antimicrobial activity. The clinical efficacy of gamithromycin administered as a single SC dose for the treatment of bovine respiratory disease in natural rather than experimental settings supports the notion that measurement of drug concentrations at sites of infection provides more clinically relevant information than does simple reliance on plasma drug concentrations.

Results of the present study suggested that gamithromycin administered SC once to beef calves distributes rapidly and extensively to PELF, BAL cells, and lung tissue, achieving concentrations exceeding MIC₉₀ for common bacterial respiratory pathogens within 30 minutes after administration. In addition, the drug appears to be eliminated slowly from these sites. These attributes are advantageous for an antimicrobial used for the single-dose treatment of the bacterial infections associated with bovine respiratory disease.

References