Concentrations of gentamicin in serum and bronchial lavage fluid after once-daily aerosol administration to horses for seven days

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Objective—To assess gentamicin concentrations in serum and bronchial lavage fluid (BLF) of horses during a 24-hour period after once-daily aerosol administration of gentamicin (G_{AER}) for 7 days and the pattern and degree of bronchial tree inflammation associated with repeated G_{AER}.

Animals—13 healthy adult horses (9 geldings and 4 mares).

Procedure—The treatment group comprised 8 horses, and 5 horses were untreated control animals. Gentamicin (20 mL of gentamicin [50 mg/mL]) was administered via aerosol once daily for 7 days. Samples of serum and BLF were obtained from all horses before G_{AER} and 0.5, 4, 8, and 24 hours after the final day of G_{AER}. Gentamicin concentrations were determined for all samples from treated horses, and cytologic examinations were performed on all BLF samples.

Results—Peak median BLF gentamicin concentration detected at 0.5 hours was 2.50 µg/mL. Median serum gentamicin concentration was < 0.50 µg/mL at all time points. Significant differences were not observed in total nucleated cell counts or differential cell counts in BLF between groups at any time point. Neutrophil count in BLF for all horses was increased over baseline at 4 and 24 hours.

Conclusions and Clinical Relevance—We did not detect evidence of gentamicin accumulation or respiratory inflammation after once-daily G_{AER} for 7 days. This protocol appears unlikely to result in local or systemic toxicosis. Repeated daily G_{AER} to horses appears to be a safe procedure and may have clinical use in the treatment of horses with bacterial infections of the airways. (*Am J Vet Res* 2004;65:173–178)

Aerosol administration of antimicrobials can result in high local concentrations of antimicrobial within the respiratory tract.[1-6] Aerosol administration of antimicrobials may also allow for a decrease in the total dose administered, rapid onset of action, and avoidance of systemic effects.[7,8] Aerosolized antimicrobial treatment has efficacy in decreasing the severity or duration of bacterial infections in the respiratory tract of several species[2,9-14]; however, clinical efficacy in horses remains limited to anecdotal reports.[15-17] Clinical application of antimicrobial aerosol treatment requires a protocol capable of effectively delivering the antimicrobial to the bronchial tree without causing substantial adverse effects.

In another study[3] conducted by our laboratory group, we reported the time-concentration relationships of gentamicin in **bronchial lavage fluid (BLF)** and serum after a single dose of gentamicin delivered by aerosol (10 minutes of nebulization with a solution of gentamicin [50 mg/mL] in sterile water) or IV (6.6 mg/kg) administration. In that study, we documented the fact that aerosol administration achieved gentamicin concentrations in BLF approximately 12 times greater than concentrations achieved by IV administration, which is similar to results of another study[5] of aerosolized administration of an aminoglycoside. This has potential clinical importance because the outcome of respiratory tract infections is more closely associated with antimicrobial concentrations within the airways than with concentrations in serum.[18-20] However, it has not been determined whether repeated aerosol administration of gentamicin will be associated with accumulation of gentamicin locally within the respiratory tract or systemically, potentially requiring alteration of the dosage or frequency of administration. Additionally, in the single-dose study we conducted, there was a mild inflammatory response to aerosol administration of gentamicin within the bronchial tree, consisting of increases in total nucleated and neutrophil cell counts, and there was concern that repeated aerosol administration could result in more severe inflammation of the respiratory tract. Thus, the study reported here was designed to determine whether daily aerosol administration of gentamicin would result in accumulation of gentamicin or inflammation of the smaller airways in adult horses.

Materials and Methods

Horses—Thirteen healthy adult horses (9 geldings and 4 mares) were used in the study. Gross evidence of respiratory tract disease, such as nasal discharge, labored respiration, or coughing, were the only criteria used to exclude a horse from the study. Horses were housed in small paddocks that contained open-sided sheds to provide shelter. Water and timothy hay were available at all times. The study protocol was approved by the Virginia Polytechnic and State University Animal Care and Use Committee.

Study design—Eight horses were included in the treatment group, and 5 were included in the untreated control

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group. We did not include a vehicle-treated control group. Treatments consisted of aerosol administration of gentamicin (10 minutes of nebulization with 20 mL of gentamicin solution [100 mg/mL[a] diluted to 50 mg/mL by the addition of sterile saline {0.45% NaCl} solution]). Each horse in the treatment group received aerosolized gentamicin once daily for 7 days. Horses in the control group were housed with the treated horses but were not administered aerosolized gentamicin.

Gentamicin administration—Aerosol administration of gentamicin was accomplished via a close-fitting facemask[b] placed over each horse's muzzle. The facemask contained 1 central inlet and 2 lateral exhalation valves. Flexible plastic tubing was used to connect the facemask to an ultrasonic nebulizer.[c] Twenty milliliters of dilute gentamicin solution (50 mg/mL) was placed into the disposable nebulizer cup, and the nebulizer was run for 10 minutes. Mean ± SEM volume of gentamicin solution remaining in the nebulizer cup at the completion of nebulization was 7.1 ± 0.2 mL.

Collection of BLF—Bronchial lavage was performed in all horses before aerosol administration of gentamicin and at 0.5, 4, 8, and 24 hours after aerosol administration on the final day of treatment. The timing of all sample collections was the same in treated and untreated horses. Bronchial lavage was performed with 30 mL of sterile saline (0.9% NaCl) solution delivered by bronchoscopy into specific regions of the lungs as described elsewhere.[3] Samples of BLF were processed as described elsewhere.[3]

Serum samples—Blood samples for determination of serum concentrations of gentamicin were collected from the left jugular vein into a plain vacuum collection tube immediately before each bronchial lavage procedure. Blood samples were allowed to clot and then centrifuged at 1,900 × *g* for 10 minutes at 4°C. Serum was harvested and divided into 2 aliquots, which were frozen at –70°C until gentamicin assays were performed.

Gentamicin assay—Gentamicin concentrations in BLF and serum were measured by use of a modified automated fluorescence polarization immunoassay.[d] The standard assay procedure was modified to increase assay precision.[21] The modification was used to ensure the sampling pipette did not contact the original sample; this prevented the carryover of gentamicin from samples of higher concentration to subsequently analyzed samples of lower concentration. This carryover phenomenon has been identified in similar instruments[e] and is attributable to the initialization process wherein the assay device identifies the samples to be analyzed by serially placing the pipette into each sample well.

In standard configurations, the assay device pipettes 20 µL of sample from the sample well into the predilution well and then adds 340 µL of buffered diluent, providing a 1:18 dilution.[22] A sample of the diluted solution is then pipetted into a glass cuvette, and reagents are added. Contents of the cuvette are exposed to polarized light (481 to 489 nm), and the net change in fluorescence and light attenuation of the sample is determined. This procedure was modified by manually pipetting 75 µL of buffered diluent into the sample well of the assay device and 20 µL of sample into the predilution well. The assay device then aspirated 20 µL of diluent from the sample well and added it to the predilution well, followed by the addition of 340 µL of diluent. This resulted in a final volume of 380 µL and provided a dilution ratio of 1:19. Because the sample wells did not contain sample, there was no carryover when the device placed the pipette into each sample well during the initialization process.

Standard curves were established for serum and BLF by use of samples of known gentamicin concentration (0.5, 1, 2, 4, 6, and 8 µg/mL) analyzed with the modified assay. The modified assay provided coefficients of variation within and between days of < 5% for BLF and < 10% for serum, respectively. Samples of known gentamicin concentration in serum or BLF and test calibration samples provided by the manufacturer were assayed in parallel with each batch of serum or BLF samples. The lower limit of detection of the assay was 0.5 µg/mL. Concentrations of < 0.50 µg/mL were reported as 0.00 µg/mL.

Cytologic analysis—Nucleated and differential cell counts were determined for aliquots of BLF obtained at all time points. Total nucleated cell counts and differential cell counts were determined manually, as described elsewhere.[3] Differential cell counts were reported as the number of cells per 200 cells.

Statistical analysis—Gentamicin concentrations were compared among time points within and between type of sample (ie, BLF and serum). Total and differential cell counts in BLF were compared among time points within and between treatment groups. Comparisons were made by use of a mixed-effect model, repeated-measures analysis.[f] Significance was defined as $P < 0.05$.

Results

Mean ± SEM yield of BLF was 11.4 ± 0.2 mL. This volume represented 38.0 ± 0.7% of the infused volume. Gentamicin concentrations were measured in BLF and serum following aerosol administration of gentamicin (**Table 1**). Distribution of the gentamicin concentrations in BLF was plotted (**Fig 1**). The gentamicin concentration in BLF at 0.5 hours after completion of aerosol treatments was significantly greater than that observed before treatments or at 8 and 24 hours after completion of treatments. Furthermore, the gentamicin concentration at 4 hours after completion of aerosol treatments was significantly greater than that observed before treatments or at 24 hours after completion of treatments. Serum gentamicin concentration after aerosol administration of gentamicin was < 0.50 µg/mL in all horses at all time points, except for a single sample obtained from 1 horse 8 hours after completion of aerosol treatments (0.77 µg/mL).

Total nucleated cell counts and differential cell counts of BLF were determined (**Table 2**). Total nucleated cell counts in BLF at all time points were within

Table 1—Median (range) concentrations of gentamicin* in bronchial lavage fluid (BLF) and serum samples obtained from 8 healthy adult horses before initiation of once-daily aerosol administration of gentamicin for 7 days and at various time points after the last daily aerosol administration of gentamicin

Sample time	BLF (µg/mL)	Serum (µg/mL)
Before treatments	0.00 (0.00–0.00)	0.00 (0.00–0.00)
After treatments (h)		
0.5	2.50 (1.00–3.59)[a]	0.00 (0.00–0.00)
4	1.45 (0.55–3.00)[b]	0.00 (0.00–0.00)
8	0.76 (0.00–2.60)	0.00 (0.00–0.77)
24	0.00 (0.00–0.58)	0.00 (0.00–0.00)

*Gentamicin concentration determined by use of a modified automated fluorescence polarization immunoassay with a lower limit of detection of 0.5 µg/mL. Concentrations < 0.50 µg/mL are reported as 0.00 µg/mL.

[a]Value differs significantly ($P < 0.05$) from values before initiation of treatments and at 8 and 24 hours after completion of treatments.

[b]Value differs significantly ($P < 0.05$) from values before treatments and at 24 hours after completion of treatments.

the reported[22] reference range of (mean ± SD) 782.2 ± 272.0 cells/μL for small-volume bronchial lavage, and we did not detect significant differences in total nucleated cell counts in BLF within or between treatment groups for the entire study or at any single time point. We did not detect significant differences between treatment groups at any time point for counts of neutrophils, macrophages, lymphocytes, eosinophils, basophils, or respiratory tract epithelial cells. When neutrophil counts (irrespective of treatment group) were compared over time, a significant increase was observed between baseline and 4 and 24 hours after completion of treatments. Lymphocyte counts in the aerosol-treated group were significantly lower at 4 hours after completion of treatments, compared with 8 hours after completion of treatments. When lymphocyte counts (irrespective of treatment group) were compared over time, a significant difference was observed between time points (before treatments and 0.5 hours after completion of treatments, 0.5 hours after completion of treatments and 4 hours after completion of treatments, and 4 hours after completion of treatments and 8 and 24 hours after completion of treatments); lymphocyte counts decreased from baseline to 4 hours after completion of treatments, then increased from 4 hours after completion of treatments to 24 hours after completion of treatments.

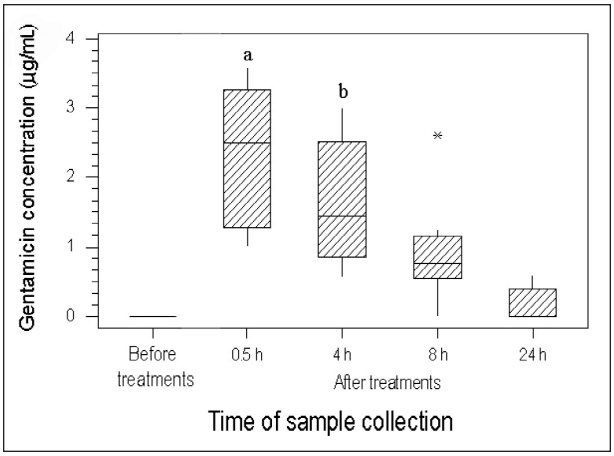

Figure 1—Box-and-whisker plots of the distribution of gentamicin concentrations in samples of bronchial lavage fluid obtained from 8 healthy horses before initiation of once-daily aerosol administration of gentamicin for 7 days and at various time points after the last daily aerosol administration of gentamicin. Box with diagonal lines represents the 25th to 75th percentiles. Horizontal line represents the median value. Whiskers represent the 10th to 90th percentiles. *Outlier data point. a—Value differs significantly ($P < 0.05$) from values before treatments and at 8 and 24 hours after treatments. b—Value differs significantly ($P < 0.05$) from values before treatments and at 24 hours after treatments.

Discussion

Successful antimicrobial treatment requires the attainment of therapeutic concentrations at the site of infection; however, many antimicrobials penetrate poorly through the respiratory tract mucosa, resulting in subtherapeutic concentrations in the pulmonary epithelial lining fluid. Aerosol administration of antimicrobials can achieve high antimicrobial concentrations in the pulmonary epithelial lining fluid or mucosa of the respiratory tract[1-6] while minimizing the development of systemic effects.[7] Aerosol administration has been considered to be incapable of delivering medication to areas of the lungs that are not ventilated; however, in 1 report,[4] even poorly ventilated or consolidated regions of the lungs contained higher antimicrobial concentrations after aerosol administration than after IV administration. Despite this finding, the

Table 2—Median (range) cell counts in samples of BLF obtained from 13 healthy adult horses (8 treated and 5 control horses) before initiation of once-daily aerosol administration of gentamicin for 7 days and at various time points after the last daily aerosol administration of gentamicin

		After treatments (h)			
Cell type	**Before treatments**	**0.5**	**4**	**8**	**24**
Total count (cells/μL)					
Treated	523 (165–909)	465 (295–909)	511 (240–1104)	617 (300–825)	461 (226–837)
Control	510 (284–602)	394 (299–584)	551 (383–792)	530 (305–795)	612 (424–669)
Neutrophils (cells/200 cells)					
Treated	6 (0–15)	19 (2–81)	19.5 (7–121)	9 (0–48)	19.5 (1–121)
Control	7 (2–10)	6 (1–28)	33 (8–58)	12 (6–34)	13 (9–54)
Macrophages (cells/200 cells)					
Treated	126 (95–158)	124 (60–137)	122 (45–164)	109 (79–140)	136 (44–145)
Control	118 (77–148)	107 (85–128)	103 (97–148)	114 (108–135)	106 (74–130)
Lymphocytes (cells/200 cells)					
Treated	47 (35–78)	40 (17–69)	28 (18–51)[a]	55 (44–84)[b]	39 (25–59)
Control	59 (14–103)	63 (49–103)	35 (24–68)	49 (41–68)	63 (51–82)
Eosinophils (cells/200 cells)					
Treated	2 (0–16)	2 (0–5)	2 (0–5)	2 (0–7)	1 (0–4)
Control	6 (1–7)	3 (0–10)	3 (0–7)	2 (0–17)	3 (0–8)
Basophils (cells/200 cells)					
Treated	0 (0–0)	0 (0–1)	0 (0–2)	0 (0–0)	0 (0–0)
Control	0 (0–1)	0 (0–0)	0 (0–0)	0 (0–0)	0 (0–0)
Respiratory tract epithelial cells (cells/200 cells)					
Treated	2 (0–26)	4 (0–20)	2 (0–8)	4 (0–10)	3 (0–7)
Control	5 (1–19)	9 (7–15)	5 (0–14)	3 (0–24)	3 (2–7)

[a,b]Within a row, values with different superscript letters differ significantly ($P < 0.05$).

administration of antimicrobials by inhalation alone is not appropriate when there is substantial consolidation or parenchymal involvement, but inhalation antimicrobial treatment may well be of benefit as an adjunct to oral or parenteral administration.

The gentamicin solution used for aerosolization in this study was diluted in a 0.45% NaCl solution because we documented in another study[3] that there was mild inflammation of the intrapulmonary conducting airways and alveoli within 24 hours after administration of a solution of gentamicin (50 mg/mL) in sterile water. This modification was made on the basis of another report[23] in which investigators concluded that antimicrobial aerosolization solutions, regardless of the specific antimicrobial, should be formulated in 0.23% to 0.45% NaCl solution to decrease the likelihood of respiratory tract inflammation and bronchoconstriction. The commercial gentamicin solution used in our formulation of the aerosolization solution contained the preservatives sodium metabisulfite, edetate disodium, methylparaben, and propylparaben, which may cause bronchoconstriction and coughing and can alter particle size and drug deposition.[24] However, a preservative-free gentamicin solution for aerosol formulation is currently not available commercially. In another report,[25] it was determined that antioxidants and preservatives in various tobramycin solutions formulated for aerosol administration were not the major cause of bronchial reactions following aerosol administration because similar responses were evident after aerosol administration of preservative-free saline solutions. We did not detect coughing or other signs of respiratory distress in any of the horses during or after nebulization in the study reported here.

A small-volume (30 mL) bronchial lavage technique was used to preferentially obtain BLF samples from the proximal portion of the bronchial tree and minimize dilution of the pulmonary epithelial lining fluid.[3] Gentamicin concentrations in BLF were reported without correcting for dilution of pulmonary epithelial lining fluid by the lavage fluid. Various techniques have been proposed for determining the volume of pulmonary epithelial fluid in BLF by correcting for the dilutional effect of the lavage fluid through the use of markers, such as endogenous albumin or urea,[26-29] or exogenous substances, such as technetium 99m Tc-pentatate, ^{51}chromium-EDTA, inulin, urea, and methylene blue.[30] Such correction techniques can introduce substantial amounts of error into the reported concentration of constituents in pulmonary epithelial lining fluid,[30,31] and for that reason, we did not use a correction technique in the study reported here. The volume of BLF obtained in this study (11.4 ± 0.2 mL) was consistent with that reported in another study[3] in which this technique was used (10.8 ± 0.2 ml), which suggests that there was minimal variation in dilution of the pulmonary epithelial lining fluid by the lavage fluid.

We did not detect evidence of gentamicin accumulation within the bronchial portion of the respiratory tract after 7 days of once-daily aerosol administration of gentamicin, as indicated by the fact that the median gentamicin concentration in BLF observed at 0.5 hours after the final dose of aerosolized gentamicin on day 7 was 2.50 µg/mL, which is lower than the peak BLF concentration reported at 0.5 hours after aerosol administration of a single dose of gentamicin in horses in another study[3] (4.47 µg/mL). This is consistent with other reports[2,6,32] of serial administration of aerosolized aminoglycosides to humans, which similarly failed to reveal gentamicin accumulation in pulmonary epithelial lining fluid or respiratory tract secretions. Although the half-life of aminoglycosides in the respiratory tract is prolonged, compared with the half-life in serum,[33,34] once-daily aerosol administration of aminoglycosides appears to allow adequate time for clearance, and longer intervals between treatments do not seem to be required.

The lower peak BLF concentration reported here was accompanied by a decrease in the range of the concentrations reported (1.00 to 3.59 µg/mL), compared with values reported in our other study[3] (3.29 to 13.34 µg/mL). This may have been associated with improvements in assay precision resulting from the described modifications to the gentamicin assay. The overall decrease in the concentration of gentamicin detected may similarly have been associated with modifications made to the assay or may have been the result of alterations in gentamicin elimination from the respiratory tract associated with repeated administration.

We did not detect evidence of gentamicin accumulation within the serum associated with repeated aerosol administration of gentamicin once daily for 7 days, and serum gentamicin concentrations were similar to those reported[3] after single-dose aerosol administration of gentamicin. This is consistent with reports[2,3,5,6,33,35-37] of serum concentrations after single or multiple doses of aerosolized aminoglycosides in several species. These results reflect the poor systemic absorption of aerosolized aminoglycosides, which reportedly[33,38] ranges from 0.3% to 2.0% or 3.3% to 14.7% of the administered dose in human patients. Because serum gentamicin concentrations of ≤ 0.5 µg/mL are below the accepted trough serum concentrations for gentamicin (ie, 1 to 2 µg/mL), the aerosol administration of gentamicin appears unlikely to interfere with the pharmacokinetics of systemically administered gentamicin or to increase the likelihood of gentamicin nephrotoxicosis.[32,38-40]

The study reported here did not yield evidence of respiratory tract inflammation associated with repeated aerosol administration of gentamicin. The only significant change observed for the aerosol-administration group was a decrease in lymphocyte count between 4 and 8 hours after completion of gentamicin administration on day 7. All other significant differences were observed for all horses in the study population, irrespective of treatment group. The lymphocyte count at 4 hours after completion of gentamicin treatments was lower than that observed before initiation of treatments or at 0.5, 8, or 24 hours after completion of treatments. The neutrophil count was greater at 4 and 24 hours after completion of treatments, compared with values obtained before initiation of treatments. This mild neutrophilic response most likely resulted from the sample collection protocol (ie, bronchial lavage) and is similar,

although of smaller magnitude, to that reported in our other study.[3] There was not a significant difference in total nucleated cell count between treatment groups or within groups over time. Total cell counts in BLF reported here, although within the reported reference ranges for small-volume bronchial lavage, were higher than those observed in our other study.[3] Because total cell counts were obtained before initiation of the gentamicin administration in the study reported here and we did not detect changes in the total cell count over time or between groups, other influences, such as environment or diet, may have been responsible.

Analysis of the results reported here supported the finding that once-daily aerosol administration of gentamicin solution (50 mg/mL) by use of an ultrasonic nebulizer is effective in delivering gentamicin to the epithelial lining fluid of the respiratory tract of horses. Repeated aerosol administration of gentamicin to horses once daily for 7 days was not associated with local or systemic accumulation of gentamicin or the development of inflammation in the bronchial tree. Clinical use of aerosol administration of gentamicin described in this report appears to be a safe procedure, but efficacy remains to be established for this treatment modality in horses.

[a]Gentozen (gentamicin sulfate solution), Schering-Plough Corp, Kenilworth, NJ.

[b]Equine Aeromask, Trudell Medical International, London, ON, Canada.

[c]UltraNeb 99, DeVilbiss, Sunrise Medical, Somerset, Pa.

[d]Abbott TDX, Abbott Laboratories, Abbott Park, Ill.

[e]Patel RR, Mrozek DC, Wiviott MM, et al. Sample cross contamination elimination with minimum wash buffer on the ARCHITECT i2000 analyzer (abstr). *Clin Chem* 1999;45(suppl 6):19.

[f]PROC MIXED, SAS, version 8, SAS Institute Inc, Cary, NC.

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