Concentrations of gentamicin in serum and bronchial lavage fluid after intravenous and aerosol administration of gentamicin to horses

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Objective—To compare concentrations of gentamicin in serum and bronchial lavage fluid after IV and aerosol administration of gentamicin to horses.

Animals—9 healthy adult horses.

Procedure—Gentamicin was administered by aerosolization (20 ml of gentamicin solution [50 mg/ml]) and IV injection (6.6 mg of gentamicin/kg of body weight) to each horse, with a minimum of 2 weeks between treatments. Samples of pulmonary epithelial lining fluid were collected by small volume (30 ml) bronchial lavage 0.5, 4, 8, and 24 hours after gentamicin administration. Serum samples were obtained at the same times. All samples were analyzed for gentamicin concentration, and cytologic examinations were performed on aliquots of bronchial lavage fluid collected at 0.5, 8, and 24 hours.

Results—Gentamicin concentrations in bronchial lavage fluid were significantly greater 0.5, 4, and 8 hours after aerosol administration, whereas serum concentrations were significantly less at all times after aerosol administration, compared with IV administration. Neutrophil counts in bronchial lavage fluid increased from 0.5 to 24 hours, regardless of route of gentamicin administration.

Conclusions and Clinical Relevance—Aerosol administration of gentamicin to healthy horses resulted in gentamicin concentrations in bronchial fluid that were significantly greater than those obtained after IV administration. A mild inflammatory cell response was associated with aerosol delivery of gentamicin and repeated bronchial lavage. Aerosol administration of gentamicin may have clinical use in the treatment of bacterial bronchopneumonia in horses. (*Am J Vet Res* 2000;61:1185–1190)

Respiratory tract disease is the second most common cause of decreased performance[1] and lost training days in horses,[2] and bacterial pneumonia has been described as the most common cause of respiratory tract disease in foals and young horses.[3] Treatment of infectious pulmonary disease has traditionally relied on oral or parenteral administration of antimicrobials. However, for antibacterial treatment to be effective, appropriate concentrations of the agent must be achieved at the site of infection.[4] One of the major limitations of oral and parenteral administration of antimicrobials is the low bioavailability of many medications in the lungs and distal airways of the respiratory tract following delivery by these routes.[5-9]

Bacterial respiratory tract infections in horses are usually precipitated by impairment of local host defenses as a result of viral infections or physiologic stress.[10,11] Thus, the primary site of infection is typically on the bronchial mucosal surface.[12] For that reason, the therapeutic outcome of respiratory tract infections is typically more closely associated with antimicrobial concentrations in airways than in serum.[5,6] Orally or parenterally administered antimicrobials may not achieve adequate concentrations within the respiratory tract, whereas aerosol administration can result in higher concentrations of antimicrobial drugs at the site of infection.[13,14] In addition, there are other potential advantages to delivering medications to the lungs and distal airways of the respiratory tract via aerosolization, including a decrease in dose administered, rapid onset of action, and avoidance or reduction of adverse effects.[15-17]

Results of several studies in humans and animals have indicated that aerosol administration of antimicrobials is effective in achieving high antimicrobial concentrations in the pulmonary epithelial lining fluid and mucosa of the respiratory tract.[13,14,18-21] Results of such studies also have indicated that aerosol administration of antimicrobials is effective in decreasing the severity or course of respiratory tract bacterial infections.[21-26] It has been suggested that aerosol administration of aminoglycosides may be of potential benefit for treatment of horses with respiratory tract infections,[27-29] but to our knowledge, there have not been studies that evaluated aerosol administration of aminoglycosides to horses. The objective of the study reported here was to compare gentamicin concentrations in serum and epithelial lining fluid obtained by lavage of the respiratory tract following aerosol and IV administration of gentamicin to adult horses. Additionally, we examined whether administration of gentamicin to the respiratory tract by aerosolization induced airway inflammation.

Materials and Methods

Horses—Nine adult horses (8 geldings, 1 mare) between 3 and 27 years old were used in the study. Horses were maintained on pasture except for the 12-hour period prior to gentamicin administration and during the sample collection period, at which time horses were housed in 12 × 12-foot box stalls and bedded on pinewood shavings. Water

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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. Gross evidence of respiratory tract disease, such as nasal discharge, labored respiration, or coughing were the only criteria for exclusion of a horse from the study.

Study design—Horses were randomly assigned to treatment groups, using a crossover design with a minimum 2-week washout period. Treatments consisted of aerosol administration of gentamicin (20 ml of a solution of 50 mg of gentamicin/ml [100 mg/ml gentamicin solution[a] diluted to 50 mg/ml with sterile water]) or IV administration of gentamicin[a] (6.6 mg/kg of body weight). Each horse received gentamicin once via each route of administration.

Gentamicin administration—Aerosol administration of gentamicin was accomplished via a close fitting face mask[b] placed over the horse's muzzle. The face mask was attached to an ultrasonic nebulizer[c] with smoothbore flexible plastic tubing. Gentamicin solution was placed into the disposable nebulizer cup, and the nebulizer was activated for 10 minutes. Gentamicin was administered intravenously via a 14-gauge, indwelling catheter[d] placed in the right jugular vein.

Bronchial lavage—To determine whether gentamicin was evenly distributed throughout the lungs after aerosol administration, 6 adult horses between 3 and 15 years old were given the same dose of gentamicin as that used in the study via face mask and nebulizer. Bronchial lavage was performed 15 minutes after administration; 4 sites (right caudoventral lung, right caudodorsal lung, left caudoventral lung, and left caudodorsal lung) were lavaged sequentially. Gentamicin concentrations were determined in each sample of lavage fluid and compared among sites by use of the Wilcoxon signed rank test. Mean (± SEM) yield of bronchial lavage fluid from all sites was 11.1 ± 0.4 ml. Mean gentamicin concentration in bronchial lavage fluid from all sites was 5.56 ± 0.61 µg/ml. We did not detect significant differences in gentamicin concentrations among sites. Because of these results, we elected to obtain samples of bronchial lavage fluid from the 9 study horses from the 4 sites sequentially, using the same order. Ventral bronchi were lavaged before dorsal bronchi on each side to avoid gravitational contamination of the ventral airways.

Horses were sedated for each lavage with acepromazine maleate (0.02 mg/kg, IV) 20 minutes prior to the procedure and xylazine hydrochloride (0.5 mg/kg, IV) immediately prior to the procedure. Bronchial lavage was performed 0.5, 4, 8, and 24 hours after gentamicin administration. A 2-meter-long video endoscope[e] was used for lavage. Prior to endoscopy, a polyethylene tube[f] with an inner diameter of 1.57 mm was inserted into the biopsy channel of the endoscope, and a 16-gauge blunt needle was inserted into the outer end of this tube to enable attachment of a Luer-tip syringe. As the endoscope was advanced down the trachea, 40 ml of a 0.2% mepivacaine solution was infused into the trachea via the polyethylene tubing to ensure adequate local anesthesia of the tracheal surface. On reaching the carina, 5 ml of a 0.4% mepivacaine solution was infused into the appropriate airway. The endoscope was withdrawn to the carina for 30 to 60 seconds to allow the mepivacaine to anesthetize the area, and the polyethylene tubing was withdrawn from the biopsy channel. The endoscope then was advanced to the desired airway, and the tip was wedged in the bronchus. A 60-ml syringe containing 30 ml of sterile saline (0.9% NaCl) solution and 30 ml of air was attached to the biopsy channel of the endoscope, and the contents were infused into the bronchus such that the air immediately followed the saline solution. This ensured that the full volume of saline solution was delivered to the airway. Aspiration was performed immediately after infusion of the syringe contents to enable us to collect as much of the infused volume of solution as possible. Samples of bronchial lavage fluid were centrifuged (1,900 × g for 10 minutes at 4 C). The supernatant was removed, filtered through a 0.2-µm polyethersulfone filter,[g] and 2 aliquots were frozen at –70 C until assays were performed.

Serum samples—Blood samples for determination of serum gentamicin concentration were collected from the left jugular vein into a plain vacuum collection tube 0.5, 4, 8, and 24 hours after gentamicin administration. After being allowed to clot, each blood sample was centrifuged (1,900 × g for 10 minutes at 4 C). Serum was removed, and 2 aliquots of serum were frozen at –70 C until gentamicin assays were performed.

Gentamicin assay—Gentamicin concentrations in bronchial lavage fluid and serum were measured by use of an automated fluorescence polarization immunoassay.[h] The use of this assay for determination of gentamicin concentrations in samples from horses has been reported.[30,31] The upper and lower limits of detection for this assay, as reported by the manufacturer, are 10.0 and 0.27 µg/ml, respectively. Gentamicin concentrations in samples were extrapolated from the calibration curve provided by the manufacturer. Accuracy of this assay for determining gentamicin concentrations in our samples was evaluated by analysis of bronchial lavage fluid and serum samples containing known quantities of gentamicin.[i] Test calibration samples provided by the manufacturer were assayed with each batch of unknown bronchial lavage fluid and serum samples.

Cytologic analysis—Nucleated and differential cell counts were determined for aliquots of bronchial lavage fluid obtained 0.5, 8, and 24 hours after gentamicin administration. Total nucleated cell counts were obtained manually, using a commercial pipetting and dilution system[j] and a Neubauer-ruled hemacytometer. Two separate counts were determined for each sample, and the results were averaged to obtain a mean value. Slides were prepared by cytocentrifugation and stained with eosin and thiazine stain for differential analysis. Each slide was analyzed microscopically, and differential cell counts were determined from evaluation of 200 nucleated cells for each sample.

Statistical analyses—Gentamicin concentration was compared between routes of administration (aerosol vs IV), among times after gentamicin administration (0.5, 4, 8, and 24 hours), and between types of sample (bronchial lavage fluid vs serum). Total and differential cell counts in bronchial lavage fluid were compared between routes of administration and among times after gentamicin administration. The Wilcoxon signed-rank test was used for all comparisons. All statistical analyses were performed, using a computerized program,[k] and significance was defined as $P < 0.05$.

Results

The mean (± SEM) yield of bronchial lavage fluid from all sites was 10.8 ml ± 0.2 ml. This volume represented 36 ± 0.6% of the infused volume. Gentamicin concentrations in bronchial lavage fluid 0.5, 4, and 8 hours after aerosol administration of gentamicin were significantly greater than concentrations at the same times after IV administration (**Table 1**). Serum gentamicin concentrations at all times following aerosol gentamicin administration were significantly less than concentrations following IV administration. Serum gentamicin concentration following aerosol genta-

Table 1—Median (range) concentrations of gentamicin (μg/ml)* in bronchial lavage fluid (BLF) and serum samples obtained at various times after aerosol and IV administration of gentamicin to 9 healthy adult horses

	Aerosol		Intravenous	
Time (h)	BLF	Serum	BLF	Serum
0.5	4.47[a] (3.29–13.34)	0.36[a] (0.27–0.45)	0.30 (0.27–2.11)	41.55 (32.90–47.60)
4	2.85[a] (0.77–5.62)	0.27[a] (0.27–0.27)	0.00 (0.27–1.80)	7.89 (5.60–9.89)
8	0.77[a] (0.62–1.03)	0.00[a] (0.00–1.19)	0.27 (0.27–0.44)	2.10 (1.17–3.24)
24	0.20 (0.00–0.27)	0.00[a] (0.00–0.27)	0.00 (0.00–0.40)	0.27 (0.00–0.44)

*Gentamicin concentration determined by use of an automated fluorescence polarization immunoassay with a lower limit of detection (accuracy) of 0.27 μg/ml. Detectable concentrations < 0.27 μg/ml are reported as 0.27 μg/ml. Nondetectable concentrations are reported as 0 μg/ml.

[a]Significantly ($P < 0.05$; Wilcoxon signed-rank test) different from value determined at same time following IV administration.

Table 2—Median (range) total (cells/μl) and nucleated cell counts (cells/200 cells) determined at various times after aerosol and IV administration of gentamicin to 9 healthy adult horses

	Time (h)		
Cell type	0.5	8	24
Total			
Aerosol	275 (110–825)	495 (220–770)	385 (275–825)
IV	220 (55–330)	220 (55–990)	385 (110–770)
Neutrophils			
Aerosol	17 (6–98)	39 (4–116)	90 (14–147)[a]
IV	18 (5–69)	17 (4–55)	58 (22–137)[a]
Macrophages			
Aerosol	128 (74–167)	120 (25–180)	81 (33–139)[a]
IV	132 (91–176)	135 (79–176)	117 (32–163)
Lymphocytes			
Aerosol	25 (1–48)	28 (7–55)	13 (10–91)
IV	30 (12–68)	39 (7–77)	29 (7–50)
Eosinophils			
Aerosol	0 (0–3)	2 (0–7)	1 (0–8)
IV	1 (0–11)	0 (0–28)	0 (0–14)
Basophils			
Aerosol	0 (0–8)	0 (0–6)	0 (0–4)
IV	0 (0–0)	0 (0–0)	0 (0–1)
Respiratory epithelial cells			
Aerosol	8 (0–72)	1 (0–11)	1 (0–9)
IV	4 (0–8)	7 (2–38)	2 (0–14)

[a]Significantly ($P < 0.05$; Wilcoxon- signed rank test) different from value determined for same group 0.5 hour after gentamicin administration. RE = Respiratory epithelial cells.

micin administration was < 0.50 μg/ml in all horses at all times, with the exception of 1 horse 8 hours after aerosol administration. We attributed this latter result to experimental error.

The total nucleated cell count in bronchial lavage fluid did not change over the course of the 24-hour sample collection period, regardless of the route of gentamicin administration (Table 2), whereas the neutrophil differential cell count did increase significantly over the course of the 24-hour sample collection period, regardless of the route of gentamicin administration. The macrophage differential cell count decreased significantly over the course of the 24-hour sample collection period following aerosol administration of gentamicin.

Discussion

Analysis of our results indicated that aerosol administration of gentamicin to healthy horses results in gentamicin concentrations in bronchial lavage fluid that, for at least the first 8 hours, are significantly greater than those obtained following IV administration. The low concentration of gentamicin in bronchial lavage fluid that we detected at all times after IV administration is consistent with reports of poor penetration of aminoglycosides into bronchial secretions following IV or IM administration.[6,16,18,32-35] A small-volume (30 ml) bronchial lavage technique was used to preferentially obtain samples of bronchial lavage fluid from the proximal portion of the bronchial tree and minimize dilution of pulmonary epithelial lining fluid.[36-38] Gentamicin concentrations in bronchial lavage fluid were reported without correcting for the dilution of pulmonary epithelial lining fluid by the lavage fluid. Several techniques have been proposed for determining the volume of pulmonary epithelial fluid in bronchoalveolar lavage fluid. These techniques use endogenous albumin, urea,[35,39-41] or exogenous substances such as technetium 99m Tc-pentatate, ^{51}Cr-EDTA, inulin, urea, and methylene blue as markers of dilution.[42] It has been documented that such correction techniques introduce substantial amounts of error into the reported concentrations of constituents of pulmonary epithelial lining fluid,[42,43] and for that reason, a correction technique was not used in the study reported here. The volumes of bronchial lavage fluid that we obtained were consistent, which suggests that there was minimal variation in the dilution of the pulmonary

epithelial lining fluid by the lavage fluid. Intravenous administration of gentamicin served as the control method for assessing the effect of bronchial lavage on pulmonary inflammation. It is possible that IV administration of gentamicin affected results of cytologic examination of the bronchial lavage fluid, but, to our knowledge, there have not been any reports indicating that respiratory inflammation or altered pulmonary mechanics develop after IV administration of gentamicin.

Gentamicin concentrations in bronchial lavage fluid after IV administration reported in our study were lower than those reported by Godber et al.[9] In that study, a paper disc absorption technique was used to obtain samples of bronchial secretions, and maximum gentamicin concentration in bronchial secretions following IV administration of a single dose of gentamicin (6.6 mg/kg) ranged from 3.67 to 5.08 μg/ml, which is approximately 8 to 11 times greater than the mean gentamicin concentration in bronchial lavage fluid we determined after IV administration of gentamicin. Because the dose of gentamicin administered was the same in both studies, this difference in results suggests that the dilutional effect of the lavage fluid in our study was approximately 10-fold. Godber et al[9] also concluded that the period during which bronchial gentamicin concentrations were greater than the minimum inhibitory concentration plus the duration of the postantibiotic effect (persistent suppression of bacterial growth following removal of the antimicrobial agent) was approximately 8 hours and that IV administration of gentamicin could not be recommended for treatment of horses with airway infections. Because the maximum gentamicin concentrations in bronchial lavage fluid following aerosol administration measured in the study reported here were approximately 12 times greater than those measured following IV administration, we believe that aerosol administration results in sufficiently high airway concentrations of gentamicin to be of clinical benefit.

Gentamicin was selected for this study, because aminoglycosides have characteristics that are potentially beneficial when administered locally. Aminoglycoside antimicrobials have concentration-dependent bactericidal activity and induce prolonged postantibiotic effects against susceptible organisms.[44] High concentrations of aminoglycosides result in more rapid and extensive bacterial killing than low concentrations and prolong the duration of the postantibiotic effect.[44] High peak concentrations (greater than 8 to 10 times the minimum inhibitory concentration) decrease the emergence of resistant strains of bacteria.[44]

Administration of medications by aerosolization and inhalation has several limitations, including the inability to deliver medication to areas of the lungs that are not ventilated, potential inactivation of the medication, time-consuming administration, potential for pulmonary tissue irritation or injury, atmospheric contamination with aerosolized medication, and potential contamination of the antimicrobial solution with microorganisms.[17,45-48] As a result of the inability of aerosol administration to deliver medication to areas of the lungs that are not ventilated, the administration of antimicrobials by inhalation alone is not appropriate when substantial consolidation or parenchymal involvement is evident, but it may be of benefit as an adjunct to oral or parenteral administration.[47,49] Aminoglycoside and β-lactam antimicrobials may be inactivated in bronchial secretions, but it is likely that this can be overcome by administration of high concentrations of these antimicrobials.[8] There are several reports that document the alteration of pulmonary mechanics in humans following aerosol administration of antimicrobials, primarily as the result of bronchoconstriction, and it has been suggested that this is the result of irritation induced by the drug, the drug carrier, or the tonicity of the solution.[50-52] Bronchial constriction resulting from aerosol administration of aminoglycoside solutions can, however, be attenuated by prior treatment with a β_2-adrenergic agonist.[51,53]

Serum concentrations of gentamicin following aerosol administration are primarily the result of pulmonary absorption.[16] Gastrointestinal absorption of ingested gentamicin is a potential source of gentamicin in serum following aerosol administration, but gentamicin is poorly absorbed from the gastrointestinal tract because of its polarity.[16] Pulmonary absorption of this drug appears to be minimal, and the low serum concentrations of gentamicin that we detected after aerosol administration are consistent with results of other studies in humans and laboratory animals.[16,18,20,54] Results of studies evaluating short- and long-term aerosol administration of aminoglycoside antimicrobials to humans indicate a lack of evidence of pulmonary, renal, or neural toxicosis.[55-58] The concurrent administration of gentamicin by the aerosol and IV routes has the potential to alter the pharmacokinetics of gentamicin in the serum. However, differences were not found in peak and trough serum aminoglycoside concentrations following IV or concurrent IV and aerosol administration of aminoglycosides to humans.[56,59]

The significant increase in neutrophil differential cell count in bronchial lavage fluid that developed during the the sample collection period suggests that there was an inflammatory response to the testing procedure. Because increases in neutrophil differential cell count were detected following both routes of gentamicin administration, this response was likely attributable to repeated bronchial lavage. This was expected, because bronchoalveolar lavage can cause mild neutrophilic pulmonary inflammation in horses.[60] The clinical relevance of this inflammatory response is unclear, because the total nucleated cell counts observed at all time points were within expected limits for small-volume (50 ml) bronchoalveolar lavage in healthy horses.[36] Although previous investigators have indicated that the preservatives contained in the gentamicin solution, such as sodium sulfite, sodium metabisulfite, and EDTA, can cause bronchoconstriction and may cause pulmonary inflammation when delivered via aerosolization,[61-63] this effect was not observed in the study reported here.

[a]Gentocin (Gentamicin Sulfate Veterinary), Schering-Plough Animal Health Corp, Kenilworth, NJ.

[b]Equine Aeromask, Canadian Monaghan Ltd, London, ON, Canada.
[c]UltraNeb 99, DeVilbiss, Sunrise Medical, Somerset, Pa.
[d]Abbocath-T, Abbot Ireland, Sligo, Ireland.
[e]Videoendoscope model No. 86200, Welch-Allyn Corp, Skaneateles, NY.
[f]Intramedic, Becton Dickinson Co, Franklin Lakes, NJ.
[g]Gelman Supor 200, Gelman Sciences Inc, Ann Arbor, Mich.
[h]Abbot TdX, Abbott Laboratories, Abbott Park, Ill.
[i]Gentamicin sulfate, Sigma Chemical Co, St Louis, Mo.
[j]Unopette 5855, Becton Dickinson and Co, Franklin Lakes, NJ.
[k]Minitab for Windows, Release 10.1, Minitab Inc, State College, Pa.

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