Characterization of Antimicrobial Aerosols for Administration to Horses

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ABSTRACT

A study was conducted to characterize the aerosols produced by a medical ultrasonic nebulizer using solutions containing antimicrobials appropriate for therapy of equine lower respiratory bacterial infections (gentamicin sulfate and ceftiofur sodium). Test aerosols were generated using an ultrasonic nebulizer and were analyzed using a laser diffraction aerosol particle analyzer. The aerosol was described in terms of the particle size distribution (volume median diameter), span (sample dispersion), and aerosol density (% volume). The particle size distribution and aerosol density of gentamicin and ceftiofur aerosols were affected by the antimicrobial concentration of the solution. All solutions produced aerosols appropriate for delivery of antimicrobials to the intrathoracic airways of the horse, but the gentamicin (50 mg/ml) and ceftiofur (25 mg/ml) solutions offered the optimal combinations of particle size and aerosol density.

INTRODUCTION

The aerosol administration of antimicrobials is of interest because this method has been shown to produce high antimicrobial concentrations within the respiratory tract.1-5 Other potential advantages of aerosol administration include a decrease in the total dose administered, avoidance of systemic side effects, and rapid onset of action.6,7 Administration of antimicrobials by aerosol has been shown to be efficacious in decreasing the severity or duration of lower respiratory bacterial infections, especially in patients with chronic infections that are poorly responsive to systemic antimicrobial therapy.1,8-12 This method of administering antimicrobials to horses has been proposed by several authors,2,7,13-16 and concentrations of gentamicin achieved in bronchial lavage fluid were 12 times greater with aerosol administration than with IV administration.2 The administration of antimicrobial aerosols does have limitations, however, including potential problems with drug delivery, pulmonary irritation, and bronchoconstriction as well as the time and equipment required for administration.13,16

The pattern of deposition of aerosol particles and the efficiency of aerosol delivery are influenced by both the characteristics of the patient (tidal flow, respiratory pattern, respiratory rate, presence of respiratory disease) and those of the aerosol (aerosol particle size, aerosolized mass, tonicity, and pH of the nebulized solution). The respiratory pattern of the horse has been described as being well suited to inhalation therapy because the large tidal volume and
high flow rate enhance pulmonary deposition of aerosols. The most important aerosol characteristic is the size distribution of the aerosol particles, and this may vary significantly with different delivery systems and drug formulations. The size of the aerosol particle determines the ability of the particle to penetrate into and deposit within the respiratory tract. Particles having a diameter of 10 µm or greater are subject to deposition in the nose and nasopharynx; particles of 2 to 10 µm deposit primarily in the large airways; and particles of 0.2 to 5 µm deposit within the bronchial tree. Therefore, characterization of the aerosol is necessary to determine whether the aerosol particles produced with the particular device and solution of interest are capable of reaching the intended site of deposition. In the present study, the aerosols generated by a medical ultrasonic nebulizer were characterized using various antimicrobial solutions to determine the appropriateness of the aerosols generated for delivery of antimicrobials to the intrathoracic airways of horses.

**MATERIALS AND METHODS**

**Solutions**

The particle size distribution and aerosol density of aerosols generated by a medical ultrasonic nebulizer (DeVilbiss Ultraneb 99 Large Volume Ultrasonic, Sunrise Medical; Figure 1) were determined for an undiluted gentamicin injectable solution (100 mg/ml) (Gentocin, Schering-Plough Animal Health); gentamicin injectable solutions in sterile water (75, 50, and 25 mg/ml); gentamicin injectable solutions in 0.9% saline (75, 50, and 25 mg/ml); and ceftiofur sodium (Naxcel, Pharmacia & Upjohn Animal Health) injectable solutions in sterile water (50, 37.5, 25, and 12.5 mg/ml) (Table 1). The solution containing 50 mg gentamicin/ml in sterile water was then further characterized over the course of 10 minutes of aerosol generation.

**Aerosol Generation**

The airflow rate generated by the internal nebulizer fan at the maximum output setting, as determined using a dry test gas flow meter (Model DTM 115, Singer Corp), was 13.1 ± 0.2 L/min. For aerosol characterization, a total volume of 20 ml of test solution was placed into the disposable nebulizer cup. The nebulizer was set up with no inline valving and with the output setting on maximum. The output from the nebulizer cup was directed to the sampling area of the particle analyzer using a section of corrugated plastic nebulizer tubing (Aerosol Hose, Professional Medical) 0.91 m (36 in) long with an internal diameter of 2.2 cm (0.875 in). The nebulizer was turned on and allowed to run until a steady output of aerosol was visible in the sampling region of the particle analyzer, at which time sampling was begun.

Following completion of the initial analysis,
VMD is analogous to mass median aerodynamic diameter due to the direct relationship between volume and mass. Span is reported as a representation of the spread of the distribution of particle sizes within the sample and is similar in concept to the geometric standard deviation used in reference to particle size distributions characterized in terms of mass median aerodynamic diameter. Span is a dimensionless number and is calculated by the equation: \[ \text{Span} = \frac{D[v,0.9] - D[v,0.1]}{D[v,0.5]} \], where \( D[v,0.9] \), \( D[v,0.1] \), and \( D[v,0.5] \) are the equivalent volume diameters at 90%, 10%, and 50% cumulative volume, respectively. Aerosol density is expressed as a percent volume (i.e., the percentage of the volume of air sampled that is occupied by aerosol particles). For the further characterization of the 50-mg/ml gentamicin solution, sampling (7,500 detector sweeps) was performed every 60 sec for the duration of two separate 10-minute nebulizer runs.

**TABLE 1. Solutions Utilized in Characterization of Antimicrobial Aerosols Generated by a Medical Ultrasonic Nebulizer**

<table>
<thead>
<tr>
<th>Antimicrobial</th>
<th>Diluent</th>
<th>Drug Concentration (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gentamicin</td>
<td>0.9% Saline</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>Water</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
</tr>
<tr>
<td></td>
<td></td>
<td>75</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>None</td>
<td>100</td>
</tr>
<tr>
<td>Ceftiofur</td>
<td>Water</td>
<td>12.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>25</td>
</tr>
<tr>
<td></td>
<td></td>
<td>37.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>50</td>
</tr>
</tbody>
</table>

one gentamicin solution (50 mg/ml) in sterile water was selected for further characterization. This aerosol was selected because it had been used in an earlier equine antimicrobial aerosol administration study. The only alteration in the testing protocol for the further evaluation of the selected gentamicin solution was that the tubing used to deliver the aerosol to the sampling region was changed from corrugated to smoothbore tubing of the same inside diameter.

**Aerosol Characterization**

Particle size distribution analysis was performed using a laser diffraction aerosol particle analyzer (Malvern Mastersizer, Malvern Instruments). Each sampling consisted of 7,500 sweeps of the detector array, and each solution was sampled twice. The results of this analysis were expressed as a volume distribution, with the volume median diameter (VMD, \( D[v,0.5] \)) expressed as the diameter below or above which 50% of the volume of the aerosol resided.

**RESULTS**

The VMD, span, and aerosol density observed with the gentamicin aerosols are presented in Table 2. There was no significant effect of diluent on VMD, span, or aerosol density for gentamicin aerosols. The gentamicin aerosols demonstrated significant \( P < .05 \) decreases in VMD, span, and aerosol density with increasing gentamicin concentration, re-
Regardless of diluent, VMD ranged from 4.66 ± 0.02 µm for all of the 25-mg/ml solutions to 4.47 ± 0.19 µm for the 100-mg/ml solution (Figure 2). Span decreased from 1.41 ± 0.01 with all of the 25-mg/ml solutions to 1.2 ± 0.15 with the 100-mg/ml solution. Aerosol density ranged from 4.1 \times 10^{-3} % ± 0.2 \times 10^{-3} % for all of the 25-mg/ml gentamicin solutions to 0.4 \times 10^{-3} % ± 0.0 \times 10^{-3} % for the 100-mg/ml solution (Figure 3).

The VMD, span, and aerosol density observed with the ceftiofur aerosols are presented in Table 3. A significant (\(P < .05\)) increase was observed in VMD with increasing ceftiofur concentration (4.48 ± 0.04 µm with the 12.5-mg/ml solution to 4.63 ± 0.01 µm with the 50-mg/ml solution) (Figure 2); however, span remained unchanged. Aerosol density decreased significantly (\(P < .05\)) with increasing ceftiofur concentration, declining from 4.4 \times 10^{-3} % ± 0.1 \times 10^{-3} % with the 12.5-mg/ml solution to 3.1 \times 10^{-3} % ± 0.2 \times 10^{-3} % with the 50 mg/ml solution (Figure 3).

Further characterization of the gentamicin/water solution (50 mg/ml) yielded slightly different results than initially observed with this solution. The aerosol characteristics of this gentamicin solution over 10 min of nebulization are reported in Table 4. The mean VMD observed was 5.52 ± 0.04 µm compared with 4.54 ± 0.01 µm for the same solution in the initial analysis. The mean span was 1.87 ± 0.22 over all times compared with 1.26 ± 0.01 observed initially. Over the course of the 10-minute nebulizer run, there was a significant (\(P < .05\)) decrease in VMD from 5.74 ± 0.12 µm to 5.28 ± 0.21 µm and an increase in span from 1.69 ± 0.05 to 2.31 ± 0.28. There was no statistically significant change in aerosol density over the course of the 10-min nebulizer run.

**DISCUSSION**

The effect of an aerosolized medication is only achieved following deposition of the

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**TABLE 2. Volume Median Diameter (VMD), Span, and Aerosol Density of Gentamicin Solutions When Aerosolized from a Medical Ultrasonic Nebulizer**

<table>
<thead>
<tr>
<th>Diluent</th>
<th>Concentration (mg/ml)</th>
<th>VMD* (µm)</th>
<th>Span* (% volume)</th>
<th>Density* (% volume)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water</td>
<td>25</td>
<td>4.67 ± 0.01</td>
<td>1.41 ± 0.02</td>
<td>4.2 \times 10^{-3} ± 0.2 \times 10^{-3}</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>4.54 ± 0.01</td>
<td>1.26 ± 0.00</td>
<td>2.0 \times 10^{-3} ± 0.0 \times 10^{-3}</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>4.50 ± 0.07</td>
<td>1.21 ± 0.04</td>
<td>1.0 \times 10^{-3} ± 0.1 \times 10^{-3}</td>
</tr>
<tr>
<td>Saline</td>
<td>25</td>
<td>4.64 ± 0.04</td>
<td>1.41 ± 0.01</td>
<td>4.0 \times 10^{-3} ± 0.2 \times 10^{-3}</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>4.58 ± 0.07</td>
<td>1.25 ± 0.01</td>
<td>2.4 \times 10^{-3} ± 0.3 \times 10^{-3}</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>4.50 ± 0.04</td>
<td>1.22 ± 0.05</td>
<td>1.0 \times 10^{-3} ± 0.4 \times 10^{-3}</td>
</tr>
<tr>
<td>Pooled</td>
<td>25</td>
<td>4.66 ± 0.02</td>
<td>1.41 ± 0.01</td>
<td>4.1 \times 10^{-3} ± 0.2 \times 10^{-3}</td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>4.56 ± 0.02</td>
<td>1.26 ± 0.01*</td>
<td>2.2 \times 10^{-3} ± 0.3 \times 10^{-3a}</td>
</tr>
<tr>
<td></td>
<td>75</td>
<td>4.50 ± 0.05*</td>
<td>1.21 ± 0.01*</td>
<td>1.0 \times 10^{-3} ± 0.4 \times 10^{-3a}</td>
</tr>
<tr>
<td>None</td>
<td>100</td>
<td>4.47 ± 0.19*</td>
<td>1.23 ± 0.16</td>
<td>0.4 \times 10^{-3} ± 0.0 \times 10^{-3}</td>
</tr>
</tbody>
</table>

*Data are expressed as mean ± SD.

*Significantly different from value obtained for solution at 25 mg/ml concentration.
The deposition of particulate material in the respiratory tract follows a fundamental pattern, which is determined by the interaction between the characteristics of the aerosol itself and those of the individual inhaling the aerosol. The interaction of these factors determines the site to which medication is delivered, the dose delivered, the efficiency of delivery, and therapeutic efficacy of the treatment. The pattern of deposition of aerosol particles and the efficiency of aerosol delivery are strongly influenced by the characteristics of the aerosol itself, including the size distribution of the aerosol particles and the aerosol density.

The formulation of a therapeutic nebulization solution can have a major impact on the characteristics of the aerosol produced and on the patient’s response to aerosol administration. The concentration of solute as well as the surface tension and viscosity of the solution are of great importance because increases in one or all of these factors often leads to a decrease in aerosol output and in particle size.

The generation of therapeutic aerosols is accomplished using several different inhalation drug delivery systems, including either ultrasonic or compressed gas–driven (jet) nebulizers, metered-dose inhalers, and dry-powder inhalers. None of these systems, however, is capable of delivering aerosolized medication with high efficiency, such that less than 10% of the original dose gets deposited in the lower respiratory tract regardless of delivery system. Antimicrobial aerosols are generated from liq-
uids using nebulizers. Nebulization is considered to be the optimal method of administration when the dose to be delivered exceeds 200 µg and does not require coordination of administration and inhalation, depending only on normal tidal breathing. Nebulizers must be used with a face mask in the conscious animal patient, with attendant aerosol wastage on the face and face mask and in the nasal passages and nasopharynx. A snug-fitting, valved face mask is commercially available for the administration of aerosols to adult horses and foals (Aeromask ES, Trudell Medical International). Nebulizers are attached to the face mask using plastic aerosol tubing. Ultrasonic nebulizers are generally capable of producing more aerosol particles per unit of time than jet (air-powered) nebulizers, resulting in shorter treatment times and greater ease of use. These devices are expensive and fragile, however, limiting their clinical application outside of referral institutions. Votion and colleagues demonstrated the efficacy of one type of ultrasonic nebulizer for delivery of radioaerosol particles to the equine lung, with 5.1% of the initial dose delivered to the lungs. Viel and Tesarowski examined the radioaerosol deposition from two types of ultrasonic nebulizers in equids and found that lung deposition ranged from 0.27% to 0.33%. The aerosol characteristics of the solutions used for aerosol generation are not described in these studies, making it difficult to account for the differences in the results obtained, but it is important to note that the efficiency of delivery is typically low for nebulizer systems. This does not mean, however, that therapeutic concentrations will not be achieved. Use of an ultrasonic nebulizer and face mask system has been shown to achieve significantly higher concentrations of gentamicin within the equine lower respiratory tract than were achieved following IV administration of a 6.6-mg/kg dose, despite the fact that the dose placed within the nebulizer was based on only 2.2 mg/kg. Because the volume of fluid lining the lower respiratory tract is very small, the delivery of even a small fraction of the dose placed within the nebulizer is able to achieve very high local concentrations.

In the study reported here, the VMD of the aerosols decreased significantly with increasing gentamicin and decreasing ceftiofur concentration, but this was not significant from a clinical standpoint because the nebulizer consistently produced an aerosol with greater than 50% of the particles in the respirable range of 1- to 5-µm diameter with all of the test solutions. The significant decrease in aerosol density with increasing gentamicin or ceftiofur concentrations may have clinical relevance, however, because a decrease in the aerosol density results in a corresponding decrease in the rate of aerosol delivery. This will result in prolongation of the time required to deliver a given volume of solution. Because increases in aerosol density were correlated with decreased drug concentration, a compromise was sought between maximum drug concentration and maximum aerosol density. Based on this initiative, the solution containing 50 mg gentamicin/ml was selected for further characterization. Further characterization of the gentamicin test solution revealed a 1-µm increase in VMD and a 50% increase in span, as compared with findings in the initial analysis. The only difference in technique between these analyses was the change in the type of nebulizer tubing from corrugated to smoothbore tubing. It is likely that the corrugated tubing used in the initial portion of the study created turbulent airflow within the tubing, resulting in the increased deposition of large particles on the wall of the tubing, while more laminar airflow within the smoothbore tubing likely allowed these larger particles to reach the test chamber, resulting in a skewing of the particle size distribution to-
wards a larger VMD and span. Although this change in tubing would decrease drug losses within the tubing, it would not be expected to result in increased delivery to the intrathoracic airways because the large particles would likely be deposited in the patient’s upper respiratory tract. Over the course of the 10-minute nebulizer sequence, there was a significant decrease in VMD, most likely as a consequence of warming of the nebulizer solution or the increasing gentamicin concentration in the nebulizer solution.\textsuperscript{19,22,23,33}

The flow rate of 13 L/min generated by the fan integral to the nebulizer was used to deliver the aerosol produced within the nebulizer chamber to the sampling region of the aerosol particle analyzer. This flow rate approximates the tidal volume of a foal, but not that of an adult horse (approximately 120 L/min). Because the nebulizer does not depend on the airflow generated by the integral fan for aerosol delivery but allows for additional air to enter through the fan during inhalation, the system allows for adequate flow to meet the ventilatory requirements of adult horses.\textsuperscript{34} The flow rate passing through the nebulizer does affect the aerosol produced by the nebulizer, with higher flow rates resulting in lowering of the VMD.\textsuperscript{34–36} The decrease in the VMD likely results from increased deposition of the larger aerosol particles within the delivery tubing due to increased turbulence and inertial impaction.\textsuperscript{36} Although it was not demonstrated in this study, lowering of the VMD associated with the increased tidal flow of the adult horse may be of clinical benefit because the resulting aerosol delivered to the patient would contain fewer large particles likely to deposit within the upper respiratory tract but would have an increased percentage of particles within the respirable range of 1 to 5 µm. The ultrasonic nebulizer system examined in this study, when used in conjunction with a commercially available equine face mask, has been shown to effectively deliver aerosolized gentamicin to adult equines, achieving lower respiratory tract gentamicin concentrations approximately 12 times greater than those observed following IV administration of gentamicin at 6.6 mg/kg.\textsuperscript{2}

The type of diluent used did not have a significant effect on aerosol generation of gentamicin solutions. Ceftiofur solutions were compounded with sterile water because the compound is incompatible with saline solutions. In two studies, administration of ceftiofur nebulization to calves used a sodium ceftiofur solution containing ethanol, noting that the addition of this substance decreased the particle sizes of the aerosol to the point that almost 100% of the particles were less than 5 µm in diameter.\textsuperscript{37,38} This phenomenon may be related to a decrease in the surface tension of the

\begin{table}
\centering
\caption{Volume Median Diameter (VMD), Span, and Aerosol Density of Ceftiofur Solutions When Aerosolized from a Medical Ultrasonic Nebulizer}
\begin{tabular}{|c|c|c|c|}
\hline
Diluent & Concentration (mg/ml) & VMD (µm)* & Span* & Density* (% volume) \\
\hline
Water & 12.5 & 4.48 ± 0.04 & 1.39 ± 0.01 & 4.4×10^{-3} ± 0.1×10^{-3} \\
& 25 & 4.58 ± 0.01\textsuperscript{a} & 1.37 ± 0.01 & 3.9×10^{-3} ± 0.1×10^{-3}\textsuperscript{a} \\
& 37.5 & 4.60 ± 0.01\textsuperscript{a} & 1.33 ± 0.01 & 3.5×10^{-3} ± 0.1×10^{-3}\textsuperscript{a} \\
& 50 & 4.63 ± 0.01\textsuperscript{a} & 1.24 ± 0.09 & 3.1×10^{-3} ± 0.2×10^{-3}\textsuperscript{a} \\
\hline
\textsuperscript{a}Data are expressed as mean ± SD.
\textsuperscript{a}Significantly different from value obtained for solution at 12.5 mg/ml concentration.
\end{tabular}
\end{table}
ceftiofur solution, allowing smaller particles to form. A high surface tension of the ceftiofur solution would also explain the increase in VMD associated with increasing ceftiofur concentration observed in this study. Despite this effect, approximately 55% to 60% of the aerosol particles generated using ceftiofur in water had a diameter within the respirable range of 1 to 5 µm. The formulation of ceftiofur with ethanol may result in increased pulmonary delivery, but ethanol is not required to solubilize ceftiofur, and the presence of potentially irritating compounds in aerosol solutions should be avoided when possible.

Nebulization solutions are ideally isotonic and neutral formulations because hypertonic, hypotonic, or acidic solutions have been shown to induce coughing or bronchoconstriction or both. A recent report recommended that antimicrobial aerosolization solutions, regardless of the specific antimicrobial involved, should therefore be formulated in a saline solution of 0.23% to 0.45% concentration. In one recent study, aerosol administration of a gentamicin solution at 50 mg/ml in sterile water caused mild pulmonary inflammation within 24 hours of administration to horses. Many antimicrobial solutions marketed for IV use contain preservatives such as sodium metabisulfite, benzalkonium chloride, or EDTA, and have been shown to induce coughing and bronchoconstriction. Although no long-term adverse effects have been associated with the aerosol administration of these compounds, they should ideally be avoided, given their potential interference with efficient and consistent aerosol administration. Administration of a bronchodilator such as albuterol (Torpex, Boehringer Ingelheim Vetmedica/3M) prior to administration of potentially irritating aerosols may attenuate the bronchoconstriction and coughing associ-
ated with these aerosols and improve their pulmonary distribution.\textsuperscript{13,43,44}

\section*{CONCLUSION}

This study demonstrated that the generation of antimicrobial aerosols appropriate for treatment of equine respiratory disease is technically feasible using a medical ultrasonic nebulizer. The findings indicated that the characteristics of the aerosol produced were dependent on the specific antimicrobial compound used, and that the optimal antimicrobial concentrations for aerosolization solutions generated with the aerosolization system described would be 50 mg gentamicin/ml or 25 mg ceftiofur/ml. Ideally, the solution should be formulated with a diluent that will yield an isotonic solution, and solutions containing preservatives should be avoided. Further characterization of antimicrobial aerosols for administration to horses under conditions more representative of the respiratory parameters of the equine adult would be of interest. Additionally, characterization of the aerosols generated with antimicrobial solutions using air driven nebulizers would be of interest because these devices are less expensive and more practical for ambulatory use.

\section*{ACKNOWLEDGMENTS}

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\section*{REFERENCES}

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