Silver-Coated Endotracheal Tubes Associated With Reduced Bacterial Burden in the Lungs of Mechanically Ventilated Dogs*

Merle E. Olson, DVM, MSc; Barry G. Harmon, DVM, PhD; and Marin H. Kollef, MD, FCCP

Study objectives: To evaluate the influence of silver-coated endotracheal tubes on the lung bacterial burden of mechanically ventilated dogs.

Design: Randomized, double-blinded, controlled experiment.

Setting: Animal research facility of a regional medical university.

Patients or participants: Eleven healthy adult dogs.

Interventions: The dogs were intubated either with cuffed, noncoated endotracheal tubes or with endotracheal tubes having a novel antimicrobial silver hydrogel coating and were challenged with buccal administration of Pseudomonas aeruginosa.

Measurements and results: The silver coating delayed the appearance of bacteria on the inner surface of the endotracheal tubes ([mean ± SD] duration of mechanical ventilation before appearance of bacteria, 3.2 ± 0.8 days; mean duration of mechanical ventilation, 1.8 ± 0.4 days; p = 0.016). The mean total aerobic bacterial burden in the lung parenchyma was statistically lower among the dogs receiving the silver-coated endotracheal tubes compared to those not receiving them (4.8 ± 0.8 vs 5.4 ± 0.9 log cfu/g lung tissue, respectively; p = 0.010). Pronounced differences were seen in the gross and histologic assessments of inflammation in the lung. Using an increasing severity scale of 0 to 12 to assess four components of histology (ie, hyperemia, edema, cellular infiltration, and bacterial presence), dogs receiving noncoated endotracheal tubes had statistically greater histology scores compared to dogs receiving silver-coated endotracheal tubes (7.1 ± 1.6 vs 2.8 ± 1.2, respectively; p < 0.001).

Conclusion: These results suggest that the silver coating of endotracheal tubes may delay the onset of and decrease the severity of lung colonization by aerobic bacteria. Based on these results, clinical studies are planned to determine the safety and clinical efficacy of silver-coated endotracheal tubes in patients requiring mechanical ventilation in the ICU setting. (CHEST 2002; 121:863–870)

Key words: aspiration; critical care; endotracheal tube; mechanical ventilation; outcomes; Pseudomonas aeruginosa; silver; ventilator-associated pneumonia

Abbreviation: SDD = selective digestive decontamination

Ventilator-associated pneumonia is the most common hospital-acquired infection among patients requiring mechanical ventilation, resulting in excess mortality and prolonged lengths of hospitalization.1–6

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The colonization of the aerodigestive tract with pathogenic bacteria and the subsequent aspiration of contaminated secretions into the lower airways appears to be the most important mechanism for the development of ventilator-associated pneumonia.7
Therefore, clinical strategies aimed at preventing bacterial colonization of the host and aspiration have been most extensively investigated for the prevention of this nosocomial infection. Biofilm formation on the surface of endotracheal tubes has been implicated as an important promoter of bacterial colonization of the lower airway and as a cause of ventilator-associated pneumonia. These biofilms harbor large concentrations of bacteria that display an inherent resistance to therapy with systemically administered antibiotics. This may encourage the emergence of antibiotic-resistant bacteria within the biofilm that subsequently can colonize the respiratory tract of the patient.

The silver coating of urinary catheters has been associated with a reduced incidence of urinary tract infections. This has been hypothesized to occur by the antibacterial properties of silver and its ability to prevent biofilm formation. Therefore, we performed a prospective, double-blinded, randomized pilot investigation to test the hypothesis that the use of a silver-coated endotracheal tube could reduce the burden of aerobic bacteria within the lung parenchyma of mechanically ventilated dogs.

### MATERIALS AND METHODS

#### Study Location and Study Population

The study was conducted at the Animal Resources Center of the University of Calgary (Alberta, Canada). Twelve healthy adult dogs were employed for this investigation. This study was approved by the ethical review board of the Animal Use Committee, Faculty of Medicine, University of Calgary. The care and handling of the animals were in accordance with the guidelines and policies of good animal practice as established by the Canadian Council on Animal Care.

#### General Study Design

A prospective, double-blinded, controlled study design was employed. In this study, dogs were randomly assigned to be orally intubated either with a silver-coated endotracheal tube or a noncoated endotracheal tube. The animal care providers were blinded to the animals’ study group assignments and all interpretation of the microbiology data and histology data were performed by blinded observers. The animals were assigned to their study groups using a random-number generator. The major end point of this pilot study was the bacterial burden of the endotracheal tube surface, Airways, and lung parenchyma.

#### Endotracheal Tubes

Cuffed endotracheal tubes (Intermediate Hi-Lo [7.5 mm internal diameter]; Mallinckrodt Medical, St. Louis, MO) were used for the control animals. For the test animals, the inner and outer surfaces of identical endotracheal tubes were coated with a novel antimicrobial silver-hydrogel formulation. The tubes for the control and test groups were repackaged and sterilized by ethylene oxide so that the investigators and the animal-care providers could be blinded regarding individual animal treatment group assignments.

#### Animals

Twelve mongrel adult dogs (17 to 31 kg; Levon Thalen; Strathmore, AB, Canada) were used in the study. Six animals were assigned to receive silver-coated endotracheal tubes, while six animals received standard noncoated endotracheal tubes. All animals were healthy and free of disease prior to the initiation of the study. Animals that had received any antibiotics < 1 week prior to the study were excluded.

The animals were anesthetized with a single injection of sodium pentobarbital (30 mg/kg) and were maintained in a state of anesthesia by providing sodium pentobarbital at approximately 1 mg/kg/h. They were placed in the dorsal recumbent position for the duration of the mechanical ventilation proposed in the study protocol (ie, up to 4 days of mechanical ventilation). Animals were provided lactated Ringers solution at a rate of 100 mL/h, and urinary catheters were placed to provide urinary drainage.

Following tracheal intubation either with a noncoated or silver-coated endotracheal tube, animals were placed on a ventilator (Harvard Biosciences; South Natick, MA) set to deliver 350 to 500 mL tidal volume of room air (50% relative humidity) at a rate of 15 to 20 breaths/min. The tidal volume delivered to the animals was selected and maintained to provide peak airway pressures of < 30 cm H₂O throughout the duration of mechanical ventilation. All animals received a level of positive end-expiratory pressure of 5 cm H₂O.

#### Bacterial Challenge

Prior to the bacterial challenge, blood and buccal culture samples were taken from each animal. After sedation and tracheal intubation, each animal was challenged twice (at 1 and 8 h after the tracheal intubation) with a respiratory isolate of *Pseudomonas aeruginosa* (strain PA01). For each challenge, 5 mL of approximately 10⁷ cfu/mL of a log-phase culture of *P. aeruginosa* was instilled into the buccal pouch of the animals. The animals were positioned with their heads turned so that any excess fluid drained out from the mouth, rather than down into the pharynx.

#### Monitoring and Sampling

Buccal culture samples were taken every 24 h and were plated quantitatively on both nutrient agar and *P. aeruginosa* isolation agar to identify the total amount of aerobic bacteria and the challenge bacteria. Using sterile suctioning tubes and mucus specimen traps, animals were suctioned via the inner lumen of their endotracheal tubes three times per day to remove secretions. However, a minimal amount of recovered tracheal aspirate hindered any attempt to quantitatively assess the bacterial burden from these samples. Rather, the presence of bacteria within the endotracheal tubes was assessed by daily sampling of the endotracheal tube lumens with a cotton culture swab.

Body temperature was monitored continuously and was recorded three times daily to determine the presence of fever in the animals. Blood samples were taken daily from each animal and were cultured using an automated blood culture system (Bactec 9240; Becton-Dickinson; Franklin Lakes, NJ). The bacteria were identified as *P. aeruginosa*, other pathogenic aerobic bacteria, or contaminants, using standard microbiological methods.

#### Postmortem Examination

Animals were killed by an overdose of sodium pentobarbital after receiving 96 h of mechanical ventilation. Postmortem
Histologic Interpretation

All microscopic samples were scored based on the grading scale described below by an animal pathologist (MEO) and were scored independently by a second animal pathologist (BGH). Both pathologists were blinded to the experimental protocol and the region of sampling. The histologic classification of lung tissue specimens was similar to that employed by other investigators.  

Histologic samples of the lung were scored using several scales. 

**Hyperemia:** 0, no hyperemia; 1, (slight) capillaries distended with blood; 2, (moderate) capillaries distended with blood and some alveoli filled with serous fluid and/or blood; and 3, (severe) capillaries are distended with blood and most alveoli are filled with serous fluid and/or blood.

**Edema:** 0, no edema; 1, slight interstitial fluid accumulation; 2, serous fluid in alveoli and moderate interstitial fluid accumulation; and 3, large amounts of serous fluid in alveoli and excessive interstitial fluid accumulation.

**Cellular infiltration:** 0, no cellular infiltration into alveolar or interstitial space; 1, occasional neutrophils; lymphocytes and/or large mononuclear cells in the alveoli and interstitial space associated with some alveoli; 2, moderate numbers of neutrophils, lymphocytes, and large mononuclear cells in the alveoli and interstitial space associated with most alveoli; and 3, large numbers of neutrophils, lymphocytes, and large mononuclear cells in the alveoli and interstitial space of most alveoli.

**Bacteria:** 0, no bacteria visible; 1, occasional bacteria evident within phagocyte; 2, bacteria within most phagocytes and occasional free bacteria; and 3, large numbers of bacteria present within phagocytes and within the alveolar and interstitial spaces.

Statistical Analysis

Data were reported as the mean ± SD. All primary comparisons between the test and control animals were based on the data for each lobe, unless otherwise noted. The Fisher’s Exact Test was used to compare categoric data, and the Mann-Whitney test was used to compare non-normal, continuous data. The Spearman rank test was used to correlate histologic and microbiology data for each lobe. The $\kappa$ statistic was used to assess the interobserver agreement for lung infiltration with neutrophils.

Results

Duration of Mechanical Ventilation

Intubation was performed without difficulty and was achieved on the first attempt for all animals. Six of the animals that had received noncoated endotracheal tubes and five that had received silver-coated tubes completed the study protocol and were included in the data analysis. One animal receiving a silver-coated endotracheal tube died 6 h after intubation. This animal mistakenly received an initial tidal volume of $>500$ mL, resulting in pneumothorax and subsequent death by an overdose of sodium pentobarbital. The lungs appeared normal at necropsy, and this animal was not included in the data analysis as it did not receive the bacterial challenge with *P aeruginosa*. There was no statistical difference in the duration of mechanical ventilation and the day of death for dogs receiving either the noncoated or the silver-coated endotracheal tubes ($3.0 \pm 1.5$ vs $3.6 \pm 0.5$ days, respectively; $p = 0.330$). Three of...
five animals (60.0%) that had been treated with silver-coated endotracheal tubes survived to the end of the study period at 96 h compared to three of the six control animals (50.0%; p > 0.999). The cause of death for the dogs receiving silver-coated endotracheal tubes included euthanasia for the three dogs completing the protocol, and cardiac arrest and renal failure for the two dogs not completing the study protocol, which were expected complications among mechanically ventilated dogs. The cause of death for the dogs receiving noncoated endotracheal tubes included euthanasia for the three dogs completing the protocol, septic shock from *P. aeruginosa* bacte remia in two animals, and excessive purulent secretions resulting in endotracheal tube occlusion in one animal.

**Microbiology**

**Buccal Cultures:** For both test and control dogs, the concentration of *P. aeruginosa* in the buccal secretions increased within 24 h after anesthesia administration and inoculation to > 10^6 cfu/g aspirate. No statistical differences were seen between the two groups for the degree of buccal colonization throughout the duration of the study period.

**Endotracheal Tube Lumen Cultures:** The average time until colonization with *P. aeruginosa* of the inner lumens of the noncoated and silver-coated endotracheal tubes was 1.8 ± 0.4 and 3.2 ± 0.8 days, respectively (p = 0.016). On day 2 of mechanical ventilation, the inner lumens of six of six (100.0%) noncoated endotracheal tubes were colonized by aerobic bacteria and 1 of 5 (20.0%) silver-coated endotracheal tubes were colonized with aerobic bacteria (p = 0.015). Three of six noncoated endotracheal tubes (50.0%) and one of five silver-coated endotracheal tubes (20.0%) were colonized with *P. aeruginosa* on day 2 of mechanical ventilation (p = 0.546). Figure 1 shows the cumulative probability of the endotracheal tubes having cultures negative for *P. aeruginosa* or aerobic bacteria for the 3 days following intubation and inoculation of the dogs.

The concentration of aerobic bacteria from the sampled inner lumen segments of the endotracheal tubes at the time of necropsy was statistically greater than that for the noncoated endotracheal tubes compared to the silver-coated endotracheal tubes (6.1 ± 1.3 vs. 4.1 ± 2.1 log cfu/cm, respectively; p = 0.009). Similarly, the concentration of *P. aeruginosa* from the sampled inner lumen segments of the endotracheal tubes was greater for the noncoated endotracheal tubes (4.1 ± 1.0 vs. 2.6 ± 1.9 log cfu/cm, respectively; p = 0.076).

**Tracheal and Bronchial Cultures:** The trachea and mainstem bronchi were heavily colonized with *P. aeruginosa* at postmortem examination (Table 1). The upper, mid-portion, and distal trachea, and the mainstem bronchi were more heavily colonized with *P. aeruginosa*, and all aerobic bacteria, among dogs receiving noncoated endotracheal tubes compared to dogs receiving silver-coated endotracheal tubes.

### Table 1—**Bacterial Counts in the Trachea and Mainstem Bronchi**

<table>
<thead>
<tr>
<th>Location</th>
<th>Dogs Receiving Noncoated Endotracheal Tubes</th>
<th>Dogs Receiving Silver-Coated Endotracheal Tubes</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Proximal trachea</td>
<td>6.2 ± 0.8</td>
<td>5.5 ± 0.7</td>
<td>0.234</td>
</tr>
<tr>
<td>Mid-trachea</td>
<td>5.8 ± 0.6</td>
<td>5.3 ± 0.7</td>
<td>0.272</td>
</tr>
<tr>
<td>Distal trachea</td>
<td>5.8 ± 0.7</td>
<td>4.8 ± 0.8</td>
<td>0.054</td>
</tr>
<tr>
<td>Mainstem bronchi</td>
<td>4.4 ± 2.4</td>
<td>3.6 ± 2.0</td>
<td>0.111</td>
</tr>
</tbody>
</table>

*Values given as mean log cfu/g tissue (± SD), unless otherwise indicated.
dogs receiving silver-coated endotracheal tubes (Table 1). However, these differences did not reach statistical significance.

**Lung Parenchymal Cultures:** Bacteria were cultured from all lung tissue samples that were taken at necropsy. The total aerobic bacterial burden in the lung parenchyma was statistically lower among the dogs that had received the silver-coated endotracheal tubes (4.8 ± 0.8 vs 5.4 ± 0.9 log cfu/g lung tissue, respectively; p = 0.010) (Fig 2). The tissue concentration of *P. aeruginosa* among dogs in the silver-coated group was also lower compared to dogs in the noncoated endotracheal tube group (4.3 ± 1.2 vs 4.4 ± 2.1 log cfu/g lung tissue, respectively; p = 0.055). The achieved thresholds of *P. aeruginosa* among the 36 lung lobes from dogs receiving noncoated endotracheal tubes were 29 (80.6%) with ≥ 10^4 cfu/g, 19 (52.8%) with ≥ 10^5 cfu/g, and 6 (16.7%) with ≥ 10^6 cfu/g, compared to 20 (66.7%) with ≥ 10^4 cfu/g, 7 (23.3%) with ≥ 10^5 cfu/g, and 3 (10.0%) with ≥ 10^6 cfu/g among the 30 lung lobes from dogs receiving silver-coated endotracheal tubes (p = 0.105). The achieved aerobic bacterial thresholds among the 36 lung lobes from dogs receiving noncoated endotracheal tubes were 34 (94.4%) with ≥ 10^4 cfu/g, 24 (66.7%) with ≥ 10^5 cfu/g, and 13 (36.1%) with ≥ 10^6 cfu/g, compared to 25 (83.3%) with ≥ 10^4 cfu/g, 9 (30.0%) with ≥ 10^5 cfu/g, and 4 (13.3%) with ≥ 10^6 cfu/g among the 30 lobes from dogs receiving silver-coated endotracheal tubes (p = 0.028).

**Blood Cultures:** Positive blood cultures were seen in three of six (50.0%) control animals and in one of the five animals receiving silver-coated endotracheal tubes (20.0%; p = 0.546). *P. aeruginosa* bacteremia was seen in two of six control animals and in zero of five test animals (p = 0.455). *Staphylococcus aureus* was isolated from the blood of one dog receiving a noncoated endotracheal tube and in one dog receiving a silver-coated endotracheal tube. For the three control animals, the positive blood cultures were found on days 2, 3, and 4. The positive blood culture was seen in the test animal on day 4.

**Postmortem Examination**

**Endotracheal Tubes:** The mean score for the noncoated endotracheal tubes was statistically greater than for the silver-coated endotracheal tubes (3.6 ± 1.2 vs 1.2 ± 0.8, respectively; p = 0.030). Five of six of the noncoated endotracheal tubes (83.3%) and zero of five of the silver-coated endotracheal tubes (0.0%) had at least a 50.0% narrowing of their lumens at necropsy (p = 0.015).

**Lung:** There was no statistical difference in the mean weight of the lungs for the animals receiving the noncoated and silver-coated endotracheal tubes (634 ± 130 vs 592 ± 53 g, respectively; p = 0.524). The mean scores for the gross appearance of the entire lung for the dogs receiving noncoated and silver-coated endotracheal tubes were 3.2 ± 1.2 and 1.2 ± 0.4, respectively (p = 0.030). The major pathologic findings were congestion and hyperemia in both groups. Both veterinary pathologists found statistically significant differences in the histologic evaluation of the dogs receiving noncoated endotra-

**Figure 2.** Box plots of tissue bacterial concentrations for all aerobic bacteria and *P. aeruginosa* according to endotracheal tube coating. Boxes represent 25th to 75th percentiles with the 50th percentile (solid line) shown within the boxes. The 10th and 90th percentiles are shown as capped bars.

**Figure 3.** Scatter plot of histology scores (x-axis) plotted against the lung tissue concentration of total aerobic bacteria (y-axis). The regression line is shown.
The most prominent histologic changes consisted of diffuse neutrophil infiltration into the alveolar walls and capillaries, which was noted primarily in the dogs receiving noncoated endotracheal tubes. One observer (BGH) noted that 21 of 33 lung lobes (63.6%) from dogs receiving noncoated endotracheal tubes had large numbers of interstitial neutrophils present compared to only 1 of 28 lung lobes (3.6%) from dogs receiving silver-coated endotracheal tubes (p < 0.001). Similarly, the second observer (MEO) scored 17 of 33 lung lobes (51.5%) and none of 28 lung lobes (0.0%) from the same groups of animals, respectively, as having large numbers of interstitial neutrophils (p < 0.001). The χ² statistic for agreement between these two observers was 0.3642 (p = 0.005) for scoring neutrophil infiltration in the alveolar walls and capillaries.

**Correlation of Microbiological and Histologic Findings:** A statistically significant correlation was found between the concentration of aerobic bacteria in the lung tissue specimens and the observed histology scores (Spearman rank correlation coefficient, 0.430; p < 0.001) [Fig 3]. Similarly, a statistically significant correlation was found between the concentration of *P. aeruginosa* in the lung tissue specimens and the observed histology scores (Spearman rank correlation coefficient, 0.356; p = 0.005).

**Discussion**

We demonstrated that the use of a silver-coated endotracheal tube was associated with statistically reduced bacterial burden and lung inflammation in an animal model of ventilator-associated pneumonia. We also showed that the silver-coated endotracheal tubes were statistically less likely to become colonized with bacteria compared to noncoated endotracheal tubes. Additionally, the colonization of the inner surface of the silver-coated endotracheal tubes was statistically delayed in onset compared to the noncoated endotracheal tubes. Finally, a statistically significant correlation was found between the burden of bacteria in the lungs of the dogs and the histologic grading of inflammation.

Animal models suggest that aspirated liquid boluses contaminated with bacteria can cause pneumonia and that smaller quantities of bacteria are usually required to cause pneumonia if aspirated compared to the introduction into the lungs by the aerosol route.20,21 Similarly, host colonization with pathogenic bacteria has been shown to be an important precursor to the development of subsequent nosocomial infection, including pneumonia.22–24 Therefore, efforts directed at reducing such colonization could help to lower the rates of nosocomial infections. Silver has bactericidal characteristics, is nontoxic, does not have clinically recognized resistance among bacterial and fungal pathogens, and has been used topically to prevent infections in other settings, including burn wound infections.25,26 There also has been relatively extensive experience with silver-coated urinary catheters to prevent urinary tract infections.12–14 A recent meta-analysis27 of eight randomized controlled trials found a statistically significant reduction in the number of nosocomial infections among patients receiving silver-coated catheters. One of the most appealing properties of silver is its activity against a wide range of microorganisms, including multiresistant staphylococci and enterococci, Gram-negative enteric bacilli, *P. aeruginosa*, and *Candida albicans*.14

Previous attempts at preventing colonization of the aerodigestive tract with pathogenic bacteria have met with varying degrees of success. Selective digestive decontamination (SDD) is the application of a topical paste into the aerodigestive tract in combination with a parenteral antibiotic having activity against Gram-negative bacteria. Two meta-analyses28,29 have demonstrated that the use of SDD can prevent the development of ventilator-associated pneumonia and, possibly, can reduce hospital mortality among selected groups of patients. However, the emergence of resistance to the antibiotics employed in SDD regimens has limited its overall utilization.30 Other strategies to prevent colonization of the aerodigestive tract with pathogenic bacteria include the use of therapy with sucralfate instead of antacids and histamine type-2 receptor antagonists, the acidification of enteral feedings, the topical application of chlorhexidine, and the use of therapy with aerosolized antibiotics.7 Unfortunately, due to associated toxicity, the emergence of bacterial resistance, or a lack of clinically meaningful efficacy, these interventions have had limited success. The silver coating of endotracheal tubes may offer some potential advantages over these earlier interventions, if it can be demonstrated to be safe and effective in clinical studies. These advantages include the absence of rapid bacterial resistance to silver and the minimal additional time or labor requirements on the part of patients’ health-care providers when applying this technology.12

This study has several important limitations. First, it was performed in an animal model employing *P. aeruginosa* for the bacterial challenge. Therefore, the results may not be applicable in the clinical
setting or for infection with other bacterial species. Second, the dogs were ventilated with tidal volumes that could potentially result in ventilator-induced lung injury, although the peak airway pressures were kept to < 30 cm H₂O to prevent such injury. Nevertheless, the presence of mechanical ventilation confounds our model and likely impacted on the pathologic findings as well as the occurrence of bacterial translocation. However, this model of pneumonia more closely parallels the clinical situation in patients receiving mechanical ventilation compared to a nonventilated animal model of pneumonia. Last, despite demonstrating statistically significant differences between the treatment groups, the small sample size employed in this study limits the power of our observations. Future studies are required to reproduce these results and, more importantly, to demonstrate the clinical relevance of reducing the bacterial burden within the lungs of ventilated patients.

In summary, this preliminary study suggests that the silver coating of endotracheal tubes may be beneficial for the prevention of ventilator-associated pneumonia. Similar results have been observed in patients with urinary tract infections following the use of silver-coated urinary catheters. Based on these observations, clinical studies are planned in the ICU setting to determine the safety and efficacy of silver-coated endotracheal tubes for the prevention of hospital-acquired infections. These studies need to determine whether clinically relevant end points, such as the occurrence of ventilator-associated pneumonia, the utilization of antibiotic treatment for pneumonia, and the duration of mechanical ventilation, are impacted by the use of silver-coated endotracheal tubes. Such studies are necessary to demonstrate whether reductions in bacterial burden within the lung result in the prevention of clinically important infections that usually require antibiotic treatment.

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