INNATE IMMUNITY MEDIATING INFLAMMATION SECONDARY TO ENDOTRACHEAL INTUBATION

Carlos A. Puyo, MD; Thomas E. Dahms, PhD

**Objective:** To investigate the inflammatory markers associated with short-term endotracheal intubation in healthy surgical patients.

**Design:** An observational and prospective study of subjects scheduled for same-day surgery procedures.

**Setting:** Level I trauma center.

**Patients:** Fourteen healthy patients intubated for same-day surgery procedures. The median duration of surgery was 3 hours.

**Interventions:** Serial lavages above the tracheal cuff were obtained at the beginning of surgery, at 1 hour, and at the end of surgery; samples were assayed for cellular counts and levels of cytokines and complement 5a (C5a).

**Results:** The total number of polymorphonuclear cells (PMNs) increased almost 10-fold from intubation to extubation ($P<.01$). The levels of 3 of the cytokines measured in tracheal lavage supernatants were significantly elevated over the time of intubation: tumor necrosis factor (TNF) ($P<.01$), interleukin 6 (IL-6) ($P<.01$), and IL-1β ($P<.025$). Levels of IL-8 showed an upward trend over time but were not significantly increased; C5a levels were significantly elevated over time ($P<.05$).

**Conclusions:** Short-term intubation in healthy patients resulted in significant tracheal inflammation. Involvement of the innate immune system as documented in the present study provides information that may help to better understand the pathophysiologic characteristics of subglottic stenosis and other endotracheal injuries secondary to endotracheal intubation.


Endotracheal intubation is a method of airway protection that results in local inflammation and in some cases tracheal injury that may progress to subglottic stenosis. The cause of tracheal stenosis appears to be multifactorial, with prolonged intubation, traumatic intubation, and tracheostomy regarded as leading causes. Other factors involved in subglottic stenosis include gastric reflux, high tracheostomy tube insertion site, pediatric and geriatric populations, female patients, prolonged estrogen use, steroid use, severe respiratory failure, autoimmune diseases, laryngeal cancer, postradiation therapy, and laryngeal trauma. The incidence of tracheal stenosis following tracheal intubation and tracheostomy tube placement has been reported to be between 10% and 22%. One-third of stenotic lesions occur in prolonged intubation cases at the cuff site, likely related to local ischemia. No pathogenesis has been given for those cases in which subglottic stenosis develops distal or proximal to the cuff. Our research team has documented a progressive increase in levels of polymorphonuclear cells (PMNs), cytokines, and chemokines indicating tracheal inflammation in a swine model during short-term intubation. Other signs of tracheal injury due to the endotracheal tube (ETT) include erythema, swelling, tracheal denudation, and ulceration.

An organized airway inflammatory response requires participation of several cells (PMNs, airway epithelial cells, and monocytes), and their products (cytokines and chemokines). The role of PMNs as promoters of an inflammatory response has been demonstrated in several diseases (asthma and cystic fibrosis). Polymorphonuclear cells migrate to a site of injury following an orderly process of margination, rolling, adhesion, and dia-
pedesis that is mediated by interleukin 8 (IL-8) and C5a, among other mediators. Stimulated PMNs communicate with surrounding cells, and through modifications in membrane expression (clusters of differentiation), they can respond in a variety of ways including release of reactive oxygen species (ROS)⁶ and increasing C5a receptor expression.⁷ Polymorphonuclear cells have the capacity to produce several cytokines (IL-1β, tumor necrosis factor [TNF], and IL-8)⁸ that may participate in modulating inflammation and have a prominent role in innate immunity at a local level. The relationship between endotracheal intubation, tracheal inflammation, and local immune response has not been analyzed in humans.

We sought to determine whether the human trachea was capable of developing an immune inflammatory reaction to the presence of the ETT. Assessment of some of the factors involving innate immunity was carried out by using multiple tracheal lavages obtained from the subglottic region. The possible role of innate immunity in the tracheal inflammation observed was suggested in human trachea by the acute elevation of PMN, C5a, and a variety of cytokine levels measured during ETT presence.

METHODS

SUBJECT PREPARATION

We studied human subjects undergoing surgical procedures requiring endotracheal intubation for general anesthesia and who were admitted to Saint Louis University Hospital for same-day surgery (Table). The study was approved by Saint Louis University institutional review board and included patients classified according the American Society of Anesthesiologists (ASA) status criteria 1, 2, and 3. Any patient with an active respiratory disease (asthma, bronchitis, or recent airway surgery) was excluded from the study. Other exclusion criteria included smoking, pregnancy, immunosuppression, or inability to understand the study purpose. Samples were excluded if the differential cell count included more than 30% oral cells. Twenty patients were enrolled, but 6 were excluded from the study (1 was found to be a smoker; 1 had a sample with >30% oral cells; and 4 had not enough sample at 1 of the measurement points). Eleven men and 3 women were included, with a mean age of 43.4 years for both groups. The control specimens were obtained from each patient at time 0.

At the discretion of the anesthesiologists of record, an ETT was chosen to provide general anesthesia care after evaluation of each individual patient. Mechanical ventilation was provided using a Datex-Ohmeda Aestiva/5 ventilator, set at tidal volume of 8 to 10 mL/kg with positive end expiratory pressure (PEEP) of 5 cm H₂O. The end tidal carbon dioxide level was monitored using a Philips M1026B device for airway gases monitoring, and the oxyhemoglobin saturation was maintained at standards of practice.

Anesthesia maintenance was accomplished with either isoflurane or sevoflurane and nitrous oxide to obtain minimum alveolar concentrations at or above 1, as decided by the anesthesiologist of record. After anesthesia induction, a Hi-lo Evac ETT was placed (Mallinckrodt–Tyco Healthcare), size 7.0 mm French for women and 7.5 to 8.0 mm French for men. To minimize the possible effects of cuff pressure, pressure was monitored and maintained at no more than 20 cm H₂O, as measured by a calibrated pressure gauge (Posey Cufflator No 8199; Posey Company).

TRACHEAL LAVAGE

Tracheal lavage samples were obtained by injecting and immediately suctioning 5 mL of sterile saline solution via the dorsal opening of the ETT located proximal to the cuff. The specimen collected represented an average of more than 60% of the injected solution by a push-and-suction technique, with the suction set at less than 100 mm Hg. The tracheal specimens were taken at 3 preset time intervals (immediately after intubation, after 1 hour, and at the end of the surgery). The specimen was obtained under sterile conditions with the container immediately transported on ice to our laboratory for processing.

LAVAGE PROCESSING

The recovered volume was measured and the cells resuspended before an aliquot was removed for cell count in a hemacytometer and for differential cell counting. The differential slides were made using cytocentrifugation of 100 µL of whole lavage fluid in 100 µL of Hanks Balanced Salt Solution with 1% bovine serum albumin followed by Protocol Hema 3 stain (Fisher Scientific). Two hundred cells per cytocentrifuged sample were counted for each differential. The remaining lavage fluid was centrifuged at 1500 rpm for 15 minutes at 4°C. The supernatant was divided into aliquots and frozen, and the cell pellet was resuspended in 200 µL of lysis buffer (20mM Tris pH 7.5/150mM sodium chloride, 1mM EDTA, 1mM EGTA, 1% Triton X-100, 2.5mM sodium pyrophosphate, 1mM β-glycerol-phosphate, 1mM sodium orthovanadate, 1-mg/mL leupeptin, and 1mM phenylmethylsulfonyl fluoride) and frozen in aliquots.

ASSAYS

Bicinchoninic acid reagents (Pierce) were used for protein assay in triplicate on tracheal lavage supernatants and cell pellets based on an albumin standard curve. Inflammatory mediators in lavage cells and supernatants were determined in duplicate by enzyme-linked immunosorbent assay (ELISA) for human cytokines (R&D Systems) and complement component C5a desArg (BD Biosciences) following the manufacturer’s instructions without modification. Level of C5a desArg was measured as an indicator of C5a level because C5a is rapidly cleaved in plasma to the desArg form. The minimum detectable dose for all cytokines was 1 to 10 pg/mL, and for C5a desArg was 47 pg/mL.

Table. Surgical Procedures Performed on Patients With Short-term Endotracheal Intubation

<table>
<thead>
<tr>
<th>Procedure</th>
<th>Patients, No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Open reduction internal fixation, extremity</td>
<td>5</td>
</tr>
<tr>
<td>Open reduction internal fixation, pelvis</td>
<td>1</td>
</tr>
<tr>
<td>Laparoscopic nephrectomy</td>
<td>2</td>
</tr>
<tr>
<td>Laparoscopic cholecystectomy</td>
<td>1</td>
</tr>
<tr>
<td>Laparoscopic liver resection</td>
<td>1</td>
</tr>
<tr>
<td>Frontal sinusotomy</td>
<td>1</td>
</tr>
<tr>
<td>Wrist fusion</td>
<td>1</td>
</tr>
<tr>
<td>Humerus resection</td>
<td>1</td>
</tr>
<tr>
<td>Tibial intramedullary nailing</td>
<td>1</td>
</tr>
<tr>
<td>Laparoscopic liver resection</td>
<td>1</td>
</tr>
<tr>
<td>Frontal sinusotomy</td>
<td>1</td>
</tr>
<tr>
<td>Wrist fusion</td>
<td>1</td>
</tr>
<tr>
<td>Humerus resection</td>
<td>1</td>
</tr>
<tr>
<td>Tibial intramedullary nailing</td>
<td>1</td>
</tr>
</tbody>
</table>

Procedure Patients, No.

Wrist fusion 1
Frontal sinusotomy 1
Humerus resection 1
Tibial intramedullary nailing 1

©2012 American Medical Association. All rights reserved.
Statistical Analysis

All data analyses were performed using SPSS software, version 15.0 (SPSS). Data from multiple time points were analyzed by Friedman nonparametric 2-way analysis of variance for repeated measures followed by Wilcoxon nonparametric signed rank tests for difference of each time point from time 0. A Bonferroni correction for multiple comparisons was used. Significance was set at P < .025. Data are expressed as median (range).

Results

Tracheal lavage cytokines

The median (range) cytokine levels noted at time 0 included IL-1β, 13 (0-92) pg/mL; IL-6, 9 (0-32) pg/mL; IL-8, 353 (0-2454) pg/mL; and TNF, 0 pg/mL. All cytokine levels tended to increase by 3.2 hours, with a noticeable elevation of IL-1β (93.5 [2-436]) (P < .025), IL-6 (121 [0-634]) (P < .01), and TNF (19 [0-690]) (P < .01). Although the median (range) IL-8 level at the end of the experiment was 2267 (0-4031) pg/mL, we could not show a conclusive statistical difference owing to the large variance (Figure 2). Total protein levels in tracheal lavage did not differ significantly over time (356 [20-1935] µg/mL at time 0; 552 [87-2160] µg/mL at 1 hour; and 644 [210-7224] µg/mL at extubation).

Complement 5a

The lavage samples were tested for C5a. Nine samples were studied for a median (range) time of 3.0 (1.5-4.0) hours, and C5a level reached 20.8 (4.8-21.4) ng/mL compared with the initial level of 1.81 (0.08-15.40) ng/mL (P < .025) (Figure 3).

Cell pellets

To determine whether cells found in tracheal lavage were a source of cytokines in the specimens, we measured the cytokine levels in the cells recovered (Figure 4). The 200 µL of lysed cell pellets at time 0 and at the end of...
surgery were assessed for median (range) levels of IL-6 (1 [0-26] pg/mL and 96 [5-469] pg/mL, respectively) (P < .01); IL-8 (2447 [77-15281] pg/mL and 8127 [565-20914] pg/mL, respectively); and total protein (811 [77-6118] pg/mL and 1819 [402-7302] pg/mL, respectively). The analysis could not be completed for other mediators owing to lack of sufficient cells to process. The total number of cells of all types washed out at time 0 was 0.91 (0.068-3.600) × 10⁶; at 3.2 hours, it was 1.37 (0.21-18.00) × 10⁶.

**COMMENT**

Tracheal inflammation secondary to ETT presence was previously documented in vivo by analysis of biochemical markers in a swine model. We hypothesized that the human trachea would react to the short-term presence of an ETT by using mechanisms typical of innate immunity similar to the ones seen in the swine model. Tracheal subglottic samples obtained from patients with no active respiratory compromise provided documentation that PMN recruitment and cytokine and C5a level elevation occurred during endotracheal intubation. The total number of PMNs per sample increased significantly, as did PMNs as a percentage of total cells over the period of intubation.

There are multiple factors involved in PMN transmigration, adhesion, and activation. Polymorphonuclear cells are known to have a short lifespan and can be recruited to a site of inflammation by locally secreted cytokines. Those cytokines may also delay PMN apoptosis, thus setting up possible production of more cytokines and chemokines, sustaining local inflammation. Elevated PMN concentration has also been correlated with the activity of IL-8 on the airway during inflammation, as seen in asthma cases. However, in the present study, the correlation over time between PMN numbers and IL-8 levels was not as strong as expected, suggesting that other chemotactic factors may be implicated in the PMN elevation observed.

Cytokines are proteins produced by a variety of cells (neutrophils, airway epithelial cells, and monocytes) in response to inflammation and infection or as a result of immune responses. Cytokine activity requires a receptor-ligand complex that can be found in immune- and non-immune-related cells. Nuclear factor κB (NF-κB) is an intracellular protein that binds to DNA and is essential for transcription of several proinflammatory cytokines (IL-1β, TNF, and IL-6) and chemokines (IL-8), all detected in the present study.

Complement 5a is present in the airways and is known to be a component of the innate immune response that influences the migration of inflammatory cells to a site of injury and after exposure to noxious stimuli; it has also been found in cases of asthma, chronic obstructive pulmonary disease, and other medical conditions.

Activation of complement may have an initial protective effect followed by a proinflammatory effect if the exposure continues. Complement 5a has been described as a chemotactic anaphylatoxin with a capacity for enhancing airway hyperreactivity and increasing vascular permeability, vasodilation, and smooth muscle contractility. We documented that C5a was present in the human trachea and that its concentration was significantly increase over time, suggesting its possible role in PMN elevation while the ETT was in place.

The trachea also contains epithelial cells, dendritic cells, and airway smooth-muscle cells capable of secreting complement, cytokines, and chemokines. Airway epithelial cells are known to be a source of IL-6 and IL-8 and may induce T-helper type 2 cells in response to infection and noxious stimuli. The levels of cytokines TNF, IL-1β, and IL-6 as well as C5a showed a significant elevation from baseline in our study. Tumor necrosis factor has been found in the cellular response to bacteria, viruses, and a variety of airborne particles. Interleukin 6 has been dubbed the transition cytokine since it appears to mediate innate and acquired immunity.

The placement of the ETT carries with it increased risk for seeding oral bacteria, local abrasion (trauma), reaction to the ETT material, and possible injury due to the cuff pressure. It is possible that introduction into the trachea of oral bacteria could result in the initial stimulus for the inflammation. We observed variable amounts of bacteria in the lavages at different times, so exclusion of bacteria as a causative factor in the inflammatory process is not possible in the present study.

Consideration must also be given to the materials found in the ETT. Di-(2-ethylhexyl)-phthalate is a known component of the ETT that appears to leach out of the ETT and has been implicated in human PMN activation.
Analysis of the cell pellets indicates that some of the IL-6 and IL-8 originates there, but the exact origin of the cytokines in tracheal lavage is yet to be determined. Some studies have documented IL-8 production by PMNs[6] and also by airway epithelial cells, which represented our 2 most common cells detected in the pellets. Interleukin 6 was also detected in the cell pellets, with the airway epithelium being the most likely source, since PMNs have not been shown to produce IL-6.23 Tracheal epithelial cells and PMNs have different origins, but both have a capacity to secrete a variety of cytokines in response to alterations in local tissues.

We documented that the human trachea is capable of responding to the presence of a foreign object by releasing markers of inflammation consistent with stimulation of the innate immune system. The increases in numbers of tracheal luminal PMNs and levels of cytokines and C5α over time suggest a progressive response to a persistent stimulus. Limited information about these markers is available in longer intubation periods. A long-term study is warranted in intubated patients to examine the immunologic changes that occur in the human trachea. Further studies are needed to determine the role of the innate immune system in the development of subglottic stenosis and other conditions related to endotracheal intubation.

Submitted for Publication: April 2, 2012; final revision received July 2, 2012; accepted July 12, 2012.

Correspondence: Carlos A. Puyo, MD, Department of Anesthesiology and Critical Care, Washington University, 660 S Euclid Ave, Campus Box 8054, St Louis, MO 63110-1093 (puyoc@wustl.edu).

Author Contributions: Dr Puyo had full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis. Study concept and design: Puyo and Dahms. Acquisition of data: Puyo and Dahms. Analysis and interpretation of data: Puyo and Dahms. Drafting of the manuscript: Puyo and Dahms. Critical revision of the manuscript for important intellectual content: Puyo and Dahms. Statistical analysis: Puyo. Obtained funding: Puyo. Administrative, technical, and material support: Dahms. Study supervision: Dahms.

Financial Disclosure: None reported.

Funding/Support: Support was provided by the Department of Anesthesiology and Critical Care, Saint Louis University.

Previous Presentations: Research from this article was presented in abstract form at the International Anesthesia Research Society Meeting; March 29 to April 1, 2008; San Francisco, California; and at the American Thoracic Society (ATS) Meeting; May 16-21, 2008; Toronto, Ontario, Canada; and the ATS Meeting; May 15-20, 2009; San Diego, California.

REFERENCES