Endotracheal Tubes for Critically Ill Patients
An In Vivo Analysis of Associated Tracheal Injury, Mucociliary Clearance, and Sealing Efficacy

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CHEST 2015; 147(5):1327-1335

e-Appendix 1.

ADDITIONAL MATERIALS AND METHODS

Animal preparation and management

Large White – Landrace pigs (37.3±3.6 Kg) were randomized to be intubated with one of the endotracheal tube (ETT) reported in the main text, induced with propofol (2.5 mg/Kg), and connected to a mechanical ventilator (SERVO-I, Maquet, NJ USA) Anesthesia was maintained through infusion of propofol (0.04-0.08 mg/Kg/min) and remifentanil (0.05-2 μg/Kg/min). Mechanical ventilation was initially set in volume-control, square-wave inspiratory flow, inspiratory fraction of oxygen 0.40, duty cycle 0.33, tidal volume 10 ml/Kg, positive end-expiratory pressure 2 cm H2O and respiratory rate adjusted to maintain PaCO2 in range. Inspiratory fraction of oxygen, positive end-expiratory pressure and respiratory rate were adjusted according to blood gases. In all animals, inspiratory gases were conditioned through a Conchatherm III heated humidifier (Hudson RCI, Temecula, CA), set to maintain the airway temperature at 37ºC. The inspiratory line was thermo-insulated with foam-rubber. The internal ETT cuff pressure was maintained at 28 cm H2O, through an external mechanical device.1,2 Endogenous pneumonia was prevented with ceftriaxone. Under strict asepsis, a surgical incision of approximately 4 cm, longitudinal to the femoral vessels, was made. The sartorius and vastus medialis were gently moved aside to get access to the femoral artery and vein. We surgically cannulated the femoral artery with a 3F polyethylene catheter (Plastimed, Prodimed, St Leu-la-Forêt, France) for systemic arterial pressure monitoring through a M11766 monitor (Hewlett-Packard, Palo Alto, CA) and collection of blood samples. We inserted into the femoral vein a 3-lumen central venous catheter (Arrow, Teleflex Medical, Ireland) for blood sampling and
infusion of drugs. A urinary catheter was inserted through mini-pelvectomy. Following surgical preparation animals were positioned in lateral – slight Trendelenburg – position and turned from one side to the other every 6 hours.

**Subglottic Secretions Drainage**

In ETTs with subglottic secretion drainage, every 6h the cuff was fully deflated and the ETT gently rotated to ensure that the evacuation port was directed toward the most dependent tracheal regions. Every 2h, patency of the suction lumen was tested, and secretions were aspirated through a 10-ml syringe. In case of resistance upon aspiration, 10 mL of air were insufflated into the suction lumen, and aspiration was attempted one additional time only.

**Tracheal Injury**

Prior to intubation, the lengths between the proximal–distal cuff’s margins and the ETT proximal opening were recorded on the bronchoscope surface with tape. This ensured bronchoscopic analysis of the tracheal region where the cuff was located. At baseline, extubation, and 24, 96h thereafter, images of the cartilaginous ventral and lateral tracheal walls were recorded through a fluorescence bronchoscope (SAFE 3000, Pentax, Germany), which comprises a class 2 laser diode (wavelength 408 nm, laser power 40mW). The bronchoscope was set to acquire simultaneous white-light and fluorescence pictures. Upon activation of the laser, normal tracheal regions appeared bluish/greenish; while, injured regions were darker and brownish. We computed, through image analysis software (ImageJ, NIH, USA), the red-to-green intensity ratio (R/G) and the green-plus-blue (G+B) intensity of the most injured region (100x100 px). Thus, tracheal injury was associated with an increase in R/G and a decrease in G+B values. All values were adjusted per baseline values as follows: [(current–baseline value)/baseline value]*100, to correct for inter-subject fluorescence variability. Saturated and underexposed areas, as well as those showing specular reflections and abnormalities (i.e. secretions) were excluded from the analysis. During the image analysis, the observers were blinded to the treatment group.

The white-light bronchoscopy picture were scored by 2 bronchoscopists blinded to treatment allocation using the following score: 0, no injury; 1, mild 2, moderate and 3, severe hyperemia, edema or discoloration without ulceration; 4, superficial and 5 deep ulceration of the mucous membrane; 6, deep ulceration of the mucous membrane with exposed cartilage.

After 96h from extubation, pigs were sacrificed, re-intubated with the same ETT and the trachea in contact with the cuff excised. The length of the excised trachea was measured, and the entire section fixed in formalin. The worst histologic injury of the first and last tracheal rings in contact with the
cuff and every other ring between these two segments, was scored by a pathologist (e-Figure 2) blinded to the ETT used (0, no injury; 1, epithelial layer compression; 2, cilia loss; 3, epithelial denudation; 4, subepithelial/glandular inflammation; 5, perichondrium inflammation). Injury of the area adjacent to the SSA opening was also studied by gross examination and microscopy.

**Mucociliary clearance**

Following 28 hours of mechanical ventilation, the animal was placed prone, and the bed oriented fully horizontal. We assessed mucociliary clearance using methods described in several previous publications. A fiberscope (FI-16bs, Pentax®, Argenteuil Cedex, France, fiberscope OD 5.2 mm working channel I.D 2.6 mm,) was inserted into the ETT and advanced 5 cm beyond the tip of the ETT. We tried to avoid all contact between the fiberscope and the tracheal mucosa. The tip of the fiberscope was directed toward the dorsal (non dependent) parts of the trachea and six tantalum disks (ESPI Metals, Ashland, OR) were insufflated through its work channel. We insufflated the disks toward the non-dependent tracheal regions to avoid mucus accumulation on the dependent regions that may be transported through gravity or ventilatory airflows. The weight of each tantalum disk was 0.8 mg and thickness and diameter were 0.1-mm and 0.6 mm, respectively. Timed serial lateral fluoroscopic images were taken through a C-arm fluoroscopy system (Siremobil Compact, Siemens® AG, Erlangen, Germany), approximately every 5 min, to compute tracheal mucus velocity through movement of the tantalum disks. Analogical fluoroscopic images were captured and digitalized via a video converter unit (ADVC110, Grass Valley™, Conflans St. Honorine, France) and dedicated software (EDIUS Neo v. 1.01, Canopus Co., Ltd., Grass Valley™, Conflans St. Honorine, France) and stored into a notebook. A ruler with radio-opaque markers was applied in close proximity of the pig’s neck to correct for magnification of the fluoroscopic images. Mucus velocity was averaged from velocities of all tracked markers. Disks were tracked as long as they remained in the visual field of the fluoroscope. The position of each tantalum disk was tracked over time and velocity computed using ImageJ (NIH, Bethesda, MD, USA) and the ImageJ plug-in MTrackJ (http://www.imagescience.org/meijering/software/mtrackj/). For analysis, a number from 0 (most dependent part of the trachea; i.e., ventral tracheal surface) to six (most nondependent; i.e., dorsal tracheal surface) was assigned to each tantalum disk to describe its vertical position within the trachea. We only analyzed disks located in the most non-dependent regions 3-6. During the analysis of the tracheal mucus velocities, the observers were blinded to treatment allocation.

**Cuff leakage**

Following 52 and 73 hours from intubation, positive end expiratory pressure was increased to 5 cmH₂O and the internal cuff pressure to 40 cm H₂O. Then, animals were placed prone, and the bed oriented 30
degrees above horizontal. We instilled 2 mL of methylene blue and 3 mL phosphate buffer solution with 1.5 μL of 2.0 μm FluoSpheres® carboxylate-modified microspheres, yellow-green fluorescent (Invitrogen™, Life Technologies, Madrid, Spain) through the ETT subglottic evacuation lumen. Then, the positive end-expiratory pressure was decreased to 0 and the internal cuff pressure to 28 cm H2O. After 1 hour from instillation, we aspirated tracheal secretions via a 10-Fr open suctioning catheter (Argyle™ Suction Catheter with Mucus Trap, 20 mL) and we grossly examined secretion for presence of methylene blue. The mucus wet-weight was recorded and collected mucus stored at -4°C. We quantified, through flow cytometry, microspheres in 2 additional mL of the instilled solution and in tracheal secretions. Tracheal secretions and the instilled solution were sent to IMT/Stason Laboratories (Irvine, CA) for counting of fluorescent microspheres using flow cytometry. Briefly, control microspheres of different colors were added to all the samples. Digestion through alkaline hydrolysis was achieved adding sodium hydroxide. Samples were left in the oven at 60 degrees overnight. Then, the sample was washed with Triton X-100, centrifuged, the supernatant removed, and deoxycholate-sodium added to complete washing. The sample was finally sonicated, filtered through 50 μm filters, centrifuged again and the remaining supernatant removed. Finally, the remaining pellet, free of debris, was transferred into the glass counting tube for microspheres quantification through flow cytometer (FACScan, Model FC, Becton Dickinson Immunocytometry Systems, Mountain View, CA USA). We computed the percentage of recovered microspheres, per gram of tracheal secretions, of the total amount of instilled microspheres.

**Weaning**

Following 76 h of mechanical ventilation, we surgically removed the arterial and urinary catheter. Then, the infusion of propofol and remifentanyl was discontinued and pigs were weaned from mechanical ventilation. Upon the beginning of spontaneous ventilation, the ventilatory mode was switched to pressure support to achieve tidal volumes of approximately 8 mL/Kg. Inspiratory fraction of oxygen was progressively reduced to maintain hemoglobin oxygen saturation ≥92%. Positive end-expiratory pressure was kept at 3 cm H2O. Animals were extubated when the animal was awake and fully able to sustain spontaneous breathing. The endotracheal tube was cleaned with sterile water and alcohol, placed into a sealing plastic bag and stored at 4 °C.

**Post-extubation handling**

Animals were housed for up to 96 hours after extubation (e-Figure 1) and were fed with food and water ad libitum. Every 12h, we monitored the status of the animals and we administered 0.3 mg of buprenorphine IM.
e-Figure 1. After 76 hours of tracheal intubation, animals were weaned, extubated and housed with food and water ad libitum.

e-Figure 2. Characteristic tracheal injury histological patterns. All tracheal sections were stained with hematoxylin and eosin. A (x400), no injury; B (x400), compression of the pseudostratified epithelial layer; C (x400), cilia loss; D (x400), epithelial denudation; E (x400), subepithelial and glandular inflammation.
**e-Figure 3.** Excised tracheal sections per treatment group. The excised tracheal section in contact with the cuff differed among groups. The shortest excised trachea was 2.6±0.4 cm in the SacettTM group. A, Ruschelit® Safety Clear Plus; B, Kimvent Microcuff; C, SealGuard EvacTM; D, SacettTM; E, TaperguardTM; F, HiLoTM; G, Sheridan HVT. NA, not available.
Reference List


