Anaesthesia Circuits, Humidity Output, and Mucociliary Structure and Function

R. D. BRANSON*, R. S. CAMPBELL†, K. DAVIS‡, D. T. POREMBAKA§

Department of Surgery, University of Cincinnati Medical Center, Ohio, U.S.A.

SUMMARY
We compared the effects of humidity delivered by the circle system at low fresh gas flows (FGF) with a conventional two-limb and coaxial circuit on the structure and function of the tracheobronchial epithelium in dogs. Animals were anesthetized and mechanically ventilated using an anesthesia ventilator to maintain normocarbia. Group I (control) animals received a FGF equal to the required minute ventilation mimicking an open circuit technique. Group II and III animals had FGF set at 20% of the required minute ventilation. Group II used a two-limb circuit and Group III used a coaxial circuit. Relative humidity and temperature of inspired gases were measured at baseline and hourly afterwards. In the first experiment, biopsies of the tracheobronchial tree were obtained bronchoscopically at baseline and then hourly for six hours. Microscopic examination of these samples allowed calculation of mean ciliary length. In the second experiment, tracheal mucus flow velocity (TMFV) was measured at baseline and hourly afterward, using a cinebroncho-fibrescopic method.

Delivered absolute humidity was greatest with low FGF and a coaxial circuit, followed by low FGF and a conventional circuit, and high FGF (15±1.4 vs 9±0.8 vs 3±0.4 mg H2O, P<0.01) after two hours. Mean cilia length (μm) and TMFV (mm/min) fell during the first hour in all three groups. At hour two TMFV returned to baseline in Group III and was significantly greater than Groups I and II (0.8±0.4 vs 8.6±1.1 vs 15.4±2.1, P<0.001). Mean ciliary length demonstrated a similar pattern with reductions from baseline in all three groups for the first two hours. Groups II and III had an increase in cilia length beginning at hour three and were both significantly greater than Group I at hours 3 through 6 (1.3±0.5 vs 3.2±1.1 vs 4.2±0.8, P<0.001).

Alterations in tracheobronchial structure and function result from exposure to dry gases and are amplified by the duration of exposure. Our findings suggest a minimum of 12 to 15 mg H2O/l is necessary to prevent these alterations. In this study, the combination of low FGF and a coaxial anesthesia circuit reached this minimum threshold more quickly than a conventional two-limb circuit.

Key Words: ANAESTHETIC TECHNIQUES: closed circuit, low flow, anaesthetic gases, humidity, ciliary epithelium, tracheal mucus flow velocity

Consequences resulting from inadequate humidification of inspired gases have been described in numerous investigations over the past three decades6. Delterious findings include morphologic damage to the tracheobronchial epithelium14, retardation of the mucociliary escalator2 and heat loss8.

Alterations in pulmonary function associated with inadequate humidity include a reduction in functional residual capacity, a decrease in pulmonary compliance, an increase in pulmonary shunt, hypoxaemia and atelectasis4. The magnitude of these effects appears to increase with duration of exposure. As such, few clinically important complications are seen following short-term anaesthesia in patients with previously normal lung function7. Humidification probably plays a more important role during long procedures in patients with active pulmonary disease. In these cases, humidification may be provided from the circle system, via a heat and moisture exchanger (HME), or a heated humidifier. The efficiency of these methods is proportional to the additional cost11.

Anaesthesia and Intensive Care, Vol. 26, No. 2, April 1998
The simplest, most cost-effective method for increasing inspired humidity includes the use of low flow anaesthesia with the circle system. Further enhancement of the moisture output of the circle system has been accomplished by using a co-axial circuit and/or by directing the fresh gas flow through the CO₂ absorber. Kleemann recently demonstrated a reduction in morphologic damage to the tracheobronchial tree by reducing the fresh gas flow (FGF) relative to the expiratory minute volume (EMV). At a FGF of 0.6 l/min and EMV of 6.0 l/min, he measured a moisture content of 20 mg H₂O/l in inspired gas after ninety minutes.

This investigation compares the moisture output of the circle system with a traditional two-limb anaesthesia circuit to a co-axial circuit and the effects on tracheobronchial morphology and tracheal mucus flow velocity (TMFV) during low flow anaesthesia in dogs.

MATERIALS AND METHODS

This study was approved by the Institutional Animal Care and Use Committee. Mongrel dogs weighing between 18 and 30 kg were anaesthetized by intravenous administration of sodium pentobarbitol (30 mg/kg). Intermittent doses of pentobarbital were administered periodically throughout the experiment to maintain adequate anaesthesia. Depth of anaesthesia was monitored using eyelid response and observation of somatic movements. Anaesthesia was sufficient to suppress any spontaneous respiratory efforts. Mechanical ventilation was provided at a tidal volume of 15 ml/kg at a respiratory rate necessary to maintain end-tidal carbon dioxide concentration (P₄ECO₂) at 35 to 40 mmHg. The inspired oxygen concentration was set at 0.40. All animals were instrumented with a central venous catheter via the femoral vein for monitoring fluid balance and core temperature via a thermistor. Intravenous fluids (lactated Ringer’s) were delivered to maintain central venous pressure (CVP) between 6 and 8 mmHg. Temperature was maintained at 36±1°C using external heating. A pulse oximetry probe was placed on the animal’s tongue for continuous monitoring of oxygen saturation.

Animals were split into groups based on the type of ventilation/humidification

Group I (n=2). The control group received ventilatory support using an anaesthesia ventilator (Ventimetre, Narco Air-Shields, Healthdyne, Habboro, PA, U.S.A.) and a conventional two-limb anaesthesia circuit. A conventional carbon dioxide absorber was used. Fresh gas flow (FGF) was set to equal the required minute ventilation. A new CO₂ absorber was used for each experiment.

Group II (n=3). These animals were ventilated using the system described for Group I animals. The only difference was the FGF was set at 20% of the required minute ventilation.

Group III (n=3). These animals were ventilated identically to those in Group II, except that a co-axial anaesthesia circuit (F-circuit, King Systems, Nobelsville, IN, U.S.A.) was used. Figure 1a and 1b depict the two circuits used during the study.

In the first experiment, serial biopsies of the tracheobronchial mucosa were obtained via bronchoscopy immediately following intubation and hourly for six hours. Biopsies were taken just below the carina, with four biopsies obtained from the right mainstem bronchus and three from the left mainstem bronchus. The biopsies were obtained in a radial fashion moving cephalad to prevent contamination of subsequent samples by blood from earlier biopsies. During the experiment P₄ECO₂, temperature, CVP, heart rate, oxygen saturation and electrocardiogram were monitored continuously. Relative humidity and temperature were measured at the endotracheal tube every 30 minutes using an electronic hygrometer and recorded. The hygrometer (Gibeck Humidity Sensor System, Gibeck, Sweden) is a capacitance device with an accuracy of ±4% relative humidity and ±1% temperature. The 90% response time for relative


Reproduced with permission of the copyright owner. Further reproduction prohibited without permission.
humidity is 1.4s and the 90% response time for temperature is less than 150 ms. The sampling rate of the device is 21 Hz. Prior to use the hygrometer was calibrated using saturated solutions of sodium chloride and lithium chloride.

Signals from the hygrometer were imported to a personal computer via an analog-to-digital converter. Real time measurements of temperature and humidity were graphically displayed and the inspired humidity measured as the mean value from the beginning of inspiration to the end of inspiration. Absolute humidity was calculated from relative humidity and temperature according to the following formula:

\[ AH = (3.939 + 0.5019T + 0.00004615T^2 + 0.0004188T^3) \times RH/100 \]

where AH is absolute humidity, T is temperature and RH is relative humidity.

Biopsy specimens were placed in a fixative and immediately sent to the laboratory. All samples were prepared for light and scanning electron microscopy. A minimum of three slides were prepared from each biopsy sample. The laboratory technician preparing the slides was blinded to the treatment group. Using image analysis software (Mocha IAS, Jandel Scientific, San Rafael, CA, U.S.A.) one of the investigators (RB) measured the area and mean length of cilia. Images were coded by the laboratory technician, thus blinding the investigator to the treatment group for each sample group. Measurements from a minimum of three slides from each sample were averaged to determine the mean ciliary length. Previous studies have used cellular morphology and graded ciliary integrity. This study is the first we are aware of, where ciliary length was used as a method of assessing humidification adequately.

In the second set of experiments, six new animals were grouped as previously described and prepared in the same fashion. Tracheal mucus flow velocity (TMFV) was measured using a cinebronchoscopic method previously described by Hirsch et al. Teflon discs 0.68 mm in diameter and 0.13 mm in thickness were placed into the bronchoscope and deposited on the tracheal mucosa in a circumferential distribution. The discs were filmed through the distal lens of the bronchoscope. As the discs moved closer to the lens, they appeared larger and served as a marker of mucociliary transport. The distance from the lens and size of the discs are initially determined. The velocity of the individual discs was calculated by dividing the change in distance by the time elapsed. Time was determined from the film frame number. A minimum of eight to ten discs were evaluated to determine the mean TMFV. The particles were followed during three points in time using four to six minutes of filming. All measurements were made by the same observer to prevent interobserver variability. Measurements were made immediately following endotracheal intubation and hourly thereafter up to six hours. At the end of all experiments, animals were sacrificed using a potassium chloride induced circulatory arrest.

Statistical analysis was performed using analysis of variance for repeated measures to determine the differences in a single variable over time. Variables at a given time point for all three groups were compared using ANOVA.

RESULTS

**Delivered Humidity**

Relative humidity and temperature measurements were used to determine absolute humidity delivered via the breathing circuit. Absolute humidity results are shown in Figure 2. Absolute humidity in Group I did not exceed 5 mg H₂O/l at any time during the experiments. Absolute humidity in Groups II and III increased with time. In Group II, absolute humidity was 8 mg H₂O/l at one hour and 15 mg H₂O/l at hour six. In Group III, absolute humidity at one hour was 13 mg H₂O/l and 23 mg H₂O/l at hour six. Mean core temperatures were not significantly different between groups. Group I mean core temperature was 35.6±0.9, Group II was 36.2±0.6, and Group III was 35.9±0.8.

**Mean Cilia Length**

The mean cilia length was not significantly different immediately after intubation in the three groups (4.7±0.5 μm vs 4.8±0.7 μm vs 5.0±0.7 μm). Figure 3 depicts the changes in cilia length over the course of the experiment. After the first hour of ven-

---

*Anesthesiology and Intensive Care, Vol. 29, No. 2, April 1998*
MUCOCILIARY STRUCTURE AND FUNCTION

**FIGURE 3:** Mean ciliary length at the study time points. Group I was significantly less than Group II and Group III at 1h, 3h through 6h ($P<0.01$). Group III was significantly greater than Group I and Group II at hours 2 through 6 ($P<0.01$). Group II was significantly greater than Group I from hours 3 through 6 ($P<0.01$).

**FIGURE 4:** Mean tracheal mucus flow velocity at the study time points. Group I was significantly less than Group II and III at 1h through 6 ($P<0.001$). Group III was significantly greater than Group II at 2 through 6 hours ($P<0.01$).

tilation, cilia length was significantly reduced in Group I compared to baseline and to Groups II and III at the same time point ($P<0.01$). After two hours, all Groups demonstrated a significantly reduced cilia length compared to baseline ($P<0.01$) and Groups I and II were significantly reduced compared to Group III ($P<0.01$). By the fourth and sixth hours, mean cilia length in Group III had returned to baseline values and was statistically greater than Groups I and II ($P<0.01$). In Group I, cilia were broken, matted together, and occasionally detached from the epithelium by hour 4. During the first two hours, Groups II and III demonstrated some flattening of cilia and cone shaped clumping. In Group III this finding was not seen after hour 3.

**Tracheal Mucus Flow Velocity**

Tracheal mucus flow velocity was not significantly different in the three groups at hour 1 (15.9±3.8 vs 16.5±3.4 vs 16.2±3.9 mm/min). In Group I, TMFV fell dramatically after one hour of breathing dry gas and was negligible at hour 3. In both Groups II and III, TMFV was significantly reduced at hour 1 compared to baseline ($P<0.01$). In Group II, the maximum decrement in TMFV occurred at hour 2, after which TMFV remained fairly constant. In Group III, the maximum decrement in TMFV occurred at hour 1. By the second hour, TMFV in Group III approached baseline values and remained stable for the duration of the experiment. TMFV during the study for each group is shown in Figure 4.

**DISCUSSION**

These findings suggest that the combination of low flow anaesthesia and a co-axial ventilator circuit offers better protection of the tracheobronchial architecture and function than low flow anaesthesia and a conventional two-limb circuit. Low flow anaesthesia with either circuit was superior to high flow anaesthesia with respect to preservation of TMFV and ciliary length. Absolute humidity was highest with the co-axial circuit, confirming results by Chalon from 1978. The preservation of tracheobronchial histology and TMFV were strongly correlated with the amount of delivered humidity. Our data suggest that restoration of TMFV and prevention of ciliary damage requires a minimum delivered absolute humidity of 15 mg H₂O/l. This value agrees with that previously published by Chalon et al. It should be noted however, that this recommendation is based on short periods of ventilation in previously healthy lungs. The value of increased humidity may only be realized during prolonged procedures in patients with pre-existing pulmonary pathology.

The untoward effects of dry gases on the structure and function of the tracheobronchial epithelium have been recognized for four decades. Dalhamn found that rats had cessation of ciliary activity after breathing dry gases for only ten minutes. Toremalm found cessation of ciliary beating after 30 minutes of artificial ventilation with room air in an in vitro preparation of rabbit trachea. When inspired gases were warmed to 30°C at approximately 80% relative
humidity, ciliary beat frequencies were relatively unchanged after 140 minutes of ventilation. Torelmalm also noted that cilia which had eased beating following exposure to dry gas regained activity when exposed to humidified gas. Numerous other authors have found similar effects of dry gases delivered to the lower airway via an endotracheal tube. The adverse effects have been shown to increase in severity with time.

Chalon et al investigated the effects of dry anaesthetic gases on the tracheobronchial epithelium of anaesthetized patients. Ciliated epithelial cells were collected during tracheal suctioning and cellular damage was assessed numerically by a point scoring system. They found that patients breathing dry gases for three hours had damage to the cilia and cellular end-plate in 39% of cells retrieved. Patients who received gases at 60% relative humidity at room temperature (22 to 26°C) did not demonstrate any evidence of cellular damage compared to baseline. This finding led Chalon et al to recommend a minimum level of humidification necessary to prevent structural changes of 12-15 mg H2O/l.

Our findings also suggest there is a minimum humidity necessary to maintain normal structural integrity as well as mucociliary action. In our study, the mean ciliary length and TMVF rates were closely related to delivered humidity. When delivered humidity was less than 12 mg H2O/l, ciliary length and TMVF were significantly reduced. The effects of humidities less than 12 mg H2O/l on ciliary length lasted approximately an hour, until humidity increased. TMVF profiles were similar, but the lag time between “insufficient” and “sufficient” humidity was smaller.

These findings support the work of Hirsch et al. Using a canine model and the same TMVF measurement technique, Hirsch and colleagues noted complete cessation of TMVF after three hours of ventilation with dry gases. After three hours of humidified gases (38°C at 100% RH), TMVF returned to baseline during the third hour. These findings suggest that the ciliated epithelium of the tracheobronchial tree, while easily damaged, has excellent recuperative capabilities. This may also explain why comparative clinical studies have failed to demonstrate any improvement in postoperative lung function between patients receiving humidification and those who did not. However, this has not been studied in patients who might be considered at risk for pulmonary complications.

The moisture output of the circle system has been extensively studied. Improvements in moisture output can be accomplished via low flow technique, use of a co-axial circuit, routing of FGF through the absorber, and insulation of the circuit. Kleemann recently demonstrated the humidity delivery of the closed circuit system using low FGF. At a minute ventilation of 6.0 l/min, absolute humidities of 14 to 21.3 mg H2O/l were seen with FGF of 6.0 to 0.6 l/minute after two hours. Kleemann utilized a dual limb circuit in these experiments. Our delivered humidity was lower than that reported by Kleeman, but our FGF was approximately 20% of the required minute ventilation compared with 10% used by Kleemann. The 10% increase in FGF in our study resulted in a delivered absolute humidity approximately 20% less than that seen by Kleemann. We believe that despite the resurgence of low flow anaesthetic techniques, fresh gas flows of 20% to 30% are more common in clinical practice.

The addition of the coaxial circuit improved delivered humidity by nearly 30% compared with the traditional two-limb circuit. This finding is in agreement with previous reports. The patient’s expired heat in the external portion of the coaxial circuit serves to insulate the inspiratory limb, allowing more moisture to be carried from the absorber to the patient. The increase in moisture output over time has also been previously reported and is due to the initial heating of the absorber. Absorber size and duration of previous use also serve to alter delivered moisture. Our study design required use of a new absorber for each animal, eliminating this as a variable in our results. We believe the coaxial circuit has the advantage of producing greater moisture output at the slightly higher flows typically used.

Other methods of increasing inspired humidity include the use of an HME or heated humidifier. Each of these devices can provide humidity more quickly than the circle system. In the case of the heated humidifier, temperatures up to 41°C at 100% relative humidity can be delivered. These devices however, add cost and complexity to the anaesthetic circuit. An HME adds deadspace, resistance, and the possibility of occlusion with secretions. The heated humidifier creates condensation which must be dealt with by the anaesthetist. Both devices can exceed the moisture delivered in this study. Our goal was to evaluate the moisture output of the circle system at low flows.

Our findings confirm the well described alterations in tracheobronchial structure and function caused by mechanical ventilation with dry gases. Similarly, our measurements suggest that a minimum moisture output of 12 to 15 mg H2O/l is necessary to
REFERENCES