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Mucociliary function deteriorates in the clinical range of inspired air temperature and humidity

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Abstract *Objective:* To test whether a reduction in air temperature within the clinical range [37 °C to 30, 100% relative humidity (RH)] altered mucus transport velocity (MTV) and ciliary beat frequency (CBF) in an in vitro ovine tracheal model. *Design:* Controlled laboratory study. *Setting:* University research laboratory. *Subjects:* Farm-reared sheep. *Interventions:* Tracheae were mounted flat in an organ bath. Krebs Henseleit bathed the serosal surface and air at 100% (RH) was passed over the mucosal surface at 4 l/min. Cilial beat frequency (CBF) was measured photo-electrically and mucus transport velocity (MTV) by timing movement. After 2 h at 37 °C (100% RH) the tissue was either maintained with those settings (controls), or the air temperature reduced to 34 °C or 30 °C. Tissue was taken for histology before and after each experiment. *Measurements and results:* CBF was 19.8±2.7 beats/s and MTV 5.7±2.6 mm/min in tissue exposed to air at 37 °C. Cilial activity continued for up to 6 h in the controls but mucus transport was more fragile. Reduction of the air temperature to either 34 °C or 30 °C led to a decrease in both CBF and MTV and, frequently, total mucociliary failure. There was a reduction in epithelial mucous cell numbers in all preparations. Tissues exposed to low temperature had additional abnormal histology. *Conclusions:* Delivery of inspired gas at 30 °C, or even 34 °C, with 100% RH may not be sufficient to prevent epithelial damage occurring during 6 h exposure.

Keywords Sheep · Trachea · Humidification · Mucosa · Epithelium

Introduction

It is accepted practice to warm and humidify inspired gases delivered to intubated patients. However, the optimal condition has yet to be established. Williams and colleagues [1] developed a model which predicts that mucosal dysfunction will occur in the lower airway unless the inspired gas is conditioned to BTPS [1]. The model further suggests that the degree of dysfunction is related to the magnitude of the deviation and the exposure time. This model has not been thoroughly tested.

In order to study the effects of temperature and humidity we have developed a new preparation using the ovine trachea, the physiology of which is well known. This preparation has been designed to represent the human airway as closely as possible and parallels other studies using bovine tracheae [2]. Our apparatus is able to provide the control necessary to examine graded responses within the clinical range of inspired gas conditions.

Materials and methods

Tracheae from sheep were cut lengthwise along the mid-line of the ventral surface and mounted flat in an organ bath on a vibration-proof table (Fig. 1). The serosal surface was bathed in recirculating, gassed, Krebs Henseleit (flow 30–50 ml/min; reservoir 2l) at 37.5 °C. Air at 100% RH (MR730; Fisher and Paykel Healthcare, Auckland, New Zealand) was passed across the epithelial surface at a flow rate of 4 l/min. The epithelium was viewed using a dissecting microscope (OPMI 1 FC, Carl Zeiss, Oberkochen, Germany) fitted with a beam splitter. Mucus transport velocity (MTV) was obtained by timing the movement of reflective particles in the mucus across a calibrated eyepiece graticule. Ciliary beat frequency (CBF) was obtained by mounting a photodiode (IPL 10530DAL, Integrated Photomatrix, Dorchester, England, UK) to the beam splitter. A Fourier analysis of the signal was carried out using ADI 'Chart' software. CBF was obtained from the peak in that spectrum.

Readings of CBF and MTV were made as soon as the tissues were mounted and then at 30-min— intervals throughout the experiment. Prior to the experimental period the gas entering the mucosal chamber was maintained at 37 °C and 100% RH for 2 h to allow the preparation to stabilise. The control activity for each preparation was the mean of the readings 1.5 h and 2 h after mounting.

Preliminary experiments were carried out to establish the longevity of the preparation. In the definitive experiments the incoming gas was either left at 37 °C (control, $n=8$), or modified to either 30 °C, saturated ($n = 5$) or 34 °C, saturated ($n = 8$) after the stabilisation period.
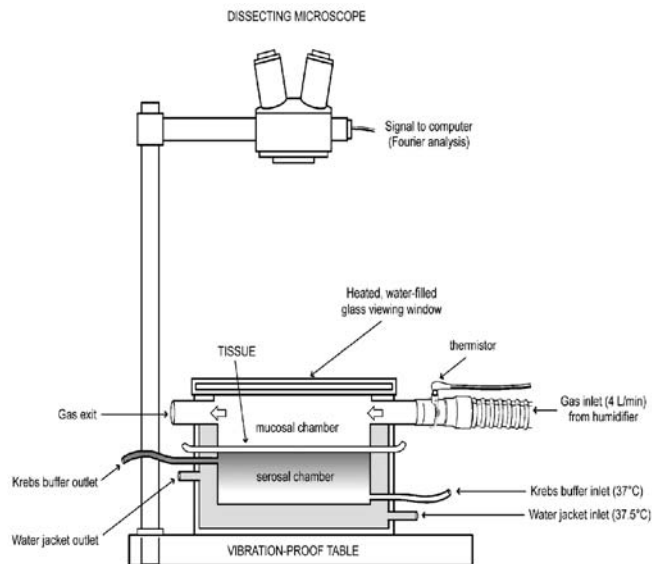

Fig. 1 The experimental apparatus. Both the organ bath and dissecting microscope were mounted on a steel plate (wt 40 kg) to avoid relative movement. This was rested on tennis balls on a 800×600×90 mm granite table. Krebs Henseleit was supplied to the serosal chamber from a 500 mL water-jacketed pressure head (not shown) the height of which could be adjusted to maintain a hydrostatic pressure across the preparation of 10–15 cmH$_2$O. A continuous flow of humidified air passed over the mucosal surface. The trachea was mounted longitudinally in the bath and the area of epithelium exposed to the airflow was 7 × 3 cm with the trachealis muscle along its centre

Histology

At the beginning and end of the experiment a piece of tracheal wall was fixed by immersion in 10% formol saline, dehydrated, embedded in paraffin wax, and 3-μm to 7-μm sections cut. These were stained with either haematoxylin and eosin, or Alcian blue (pH 2.6) and periodic acid-Schiff (PAS). Epithelial mucous cell counts were obtained and scored as either Alcian blue positive (containing acidic glycoprotein), PAS positive (containing neutral glycoprotein) or mixed. Ten fields were counted for each specimen.

Data analysis

Data were analysed using Prism software (Prism version 2.0, Graphpad Software, San Diego, Calif., US). Variations in the mean control readings were sought using one-way variance analysis and the effects of exposure times and gas environments on the mean CBF, MTV, and epithelial mucus-containing cell counts were tested using a two way analysis. Kaplan-Meier survival curves were constructed. Log-rank tests were used to compare the effects of different environments. All results are expressed as the mean±-SEM.

Results

During the stabilisation period, the CBF was 21.4±0.82 Hz, 21.3±1.1 Hz, and 22.2±1.05 Hz for the control, 34 °C, and 30 °C groups of tissue, respectively. These means were not significantly different (one way variance ratio analysis; $p = 0.84$). MTV (control, 34 °C and 30 °C) was 6.0±4.1, 11.5±1.9 and 9.5±2.4 mm/min during the control period and, again, the differences between the three groups of tissues were not significant ($P = 0.135$). Generally, there was a much greater variation between individual tissues in MTV compared with CBF (0.96 – 16.4 mm/min compared with 14.9–25.4 Hz, respectively). Because of this wide range of starting points, illustrative data (Fig. 2) is presented as percentage change from control.

Control experiments ($n = 8$)

In control experiments where the mucosal surface was exposed to air at 37 °C, 100% RH CBF was relatively stable at 19.8±2.7 beats/s (Fig. 2A). Many experiments were continued for >6 h, therefore, median survival times of the control preparations could not be calculated (survival time ≥ 6 h).

MTV was more variable at 5.7±2.6 mm/min (Fig. 2B). This variation occurred both between preparations and during the course of an individual experiment. Mucus transport was also less robust than ciliary activity, ceasing in 4/8 control preparations in ≥ 6 h. However, a sufficient number of preparations survived the experimental protocol to prevent a median survival time being calculated (survival time >6 h).
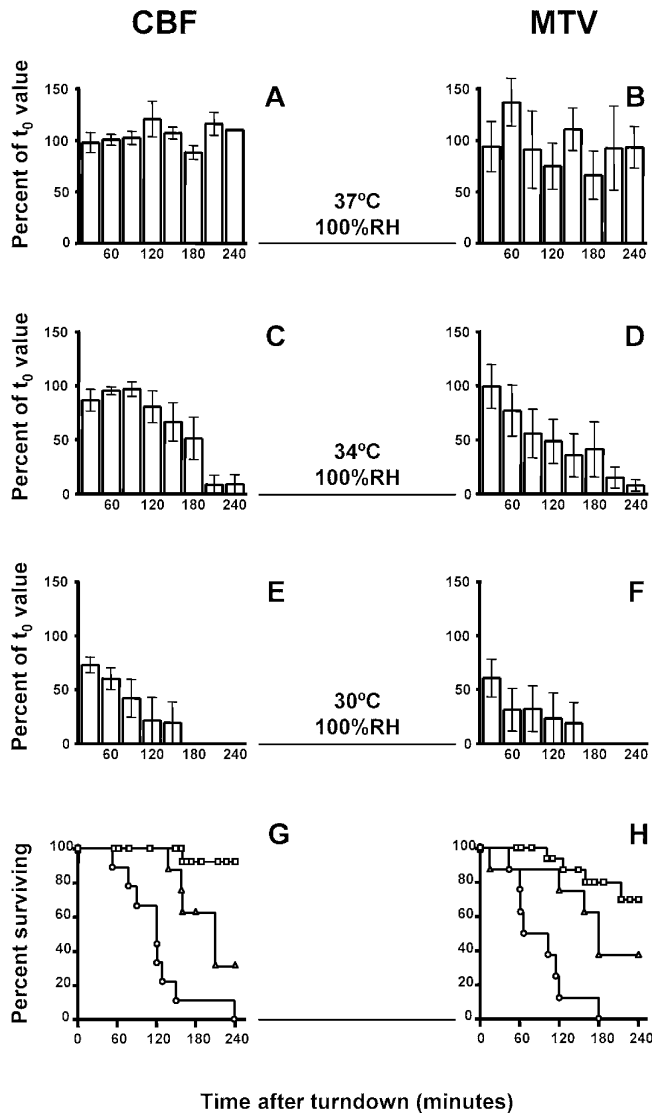
Fig. 2 The effect of lowering gas temperature on ciliary beat frequency and mucus transport velocity over time. In all experiments the gas was maintained at 100% RH. Panels A & B show that gas at 37 °C and 100%RH maintains CBF and MTV performance in the tissue preparation over the experimental time course without deterioration. In contrast, reducing the air temperature above the epithelium from 37 to 30 °C led to a rapid reduction in both MTV and CBF and the total failure of mucociliary function in all preparations within four h (panels E & F). Reduction to 34 °C produced similar changes (panels C & D) but these were less rapid. Nevertheless, this temperature caused total failure of mucociliary function in 5 of the 8 preparations. Also shown are combined survival plots for ciliary activity (panel G) and mucus transport (panel H) when the epithelium was exposed to 37 °C (☐) 34 °C (▲), and 30 °C (○). Statistical tests indicate that 34 °C and 30 °C caused a significant reduction in survival time and that the magnitude of this effect was temperature dependant

Effect of reduced temperature

When the temperature of the air was reduced to 34 °C the mean MTV (Fig. 2C) and mean CBF (Fig. 2D) decreased with time. In approximately half the preparations, MTV and CBF showed a progressive decline in velocity and rate before stopping. The median survival time of mucus movement was 180 min (range from 135 min to >240 min). The median survival time of cilial activity was 210 min (range from 138 min to >240 min).

Reduction in the temperature to 30 °C exacerbated these phenomena (Fig. 2E,F). Median survival time of mucus movement fell to 84.5 min (range 44–180 min) and ciliary activity to 120 min (range from 53 min to <240 min). CBF and MTV 'survival curve' statistics (Fig. 2G,H, respectively) were examined using the 'log-rank' survival test. Temperature reduction to 34 °C significantly reduced the survival time of MTV and CBF (P <0.0001). The log-rank test for trend indicated that this effect was graded (P <0.0001), with 30 °C producing a shorter survival time than 34 °C, but 34 °C being significant against 37 °C (survival time: 37 °C> 34 °C>30 °C).

Histology

Initially, tracheae had a near normal histology. Following a 6-h control experiment, this appearance was maintained but the number of epithelial mucus-producing cells was reduced from 52±11 cells/mm to 14±6 cells/mm.

The tissues exposed to a reduced temperature (30 °C, 100% RH) showed reduction in height, reduced number of mucus cells, apocrine secretion, and a highly Alcianophyllic disorganised and 'clumped' ciliary layer.

In a few preparations, the association between adjacent cells had failed, particularly at the apical border. There were also frequent patches of epithelium where the columnar epithelial cells had been shed, leaving only a layer of basement cells or just the basement membrane itself.

Discussion

Our results show that air temperature reduction progressively reduced MTV and CBF and often resulted in complete failure within 4 h. A reduction of temperature below control of as little as 3 °C produced a statistically significant decrease in survival time (Fig. 2G,H). These results support the predictions made by Williams et al. that deviation from BTPS would cause graded mucociliary dysfunction [1]. However, this occurred at unexpectedly high temperatures and over an unexpectedly short time.

Our preparation is not ideal in that it uses an isolated specimen which is not bathed with mucus or surfactant from the lower airways, and uses unidirectional gas flow. However, it is stable and reproducible, eliminates the confounders of the clinical context, and has been previously validated [3, 4, 5, 6].

Partial mucociliary dysfunction has been shown to be reversible but, thereafter, cilia cease beating, cell damage occurs, and function cannot be restored without epithelial regeneration [7, 8]. In several preparations cilia were observed beating below stationary mucus, suggesting that a return to the control condition may have restored complete function. The rapid subsequent halt of cilial movement suggests that the window for reversibility is small [8].

Warming the inspired air to 30 °C,100% RH reduces mucociliary dysfunction compared with dry gas. Humidification guidelines propose minimum standards to avoid epithelial damage [9, 10]. Our results, taken together with recent clinical reports [11, 12, 13], suggest that 30 °C, or even 34 °C, with 100% RH may not be sufficient to prevent epithelial dysfunction.

Clinically, humidification at the low end of the prescribed range may be sufficient to avoid mucociliary dysfunction in the short-term, but recent reports suggest that these minima may be inadequate for longer periods. Alagar et al. [12] found an increased need for saline installation and bronchodilator therapy when passive humidification was used compared with optimally humidified gas. Further, Ryan et al. [11] found a significant energy cost was incurred by the epithelium when the inspired gas was not at core temperature and saturated.

The level of humidification chosen from the recommended range will significantly effect epithelial function. This has particular relevance to patients ventilated for extended periods, to those with pre-existing disease or those with an acute airway insult. In these patients, 30 °C or even 34 °C with 100% RH may not be sufficient to prevent continued epithelial dysfunction or damage.

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