Effects of normobaric oxygen on ciliary beat frequency of human respiratory epithelium

A. STANEK, A. M. BRAMBRINK, F. LATORRE, B. BENDER AND P. P. KLEEMANN†

Summary

Respiratory infection is a major cause of morbidity after general anaesthesia. Impairment of respiratory ciliary beat frequency (CBF) by different stress factors causes a decrease in mucus transport rate (MTR). We have tested the effect of different concentrations of oxygen on CBF of human respiratory epithelium in a prospective, randomized, in vitro study. Samples of superficial mucosa of the inferior nasal turbinates of 20 non-smoking healthy volunteers were harvested and exposed to three different oxygen environments (group I=21% oxygen, group II=60% oxygen and group III=95% oxygen) for 2 h. In 50% of the samples, exposure time was prolonged. At 30, 60, 90, 120 and 240 min, light microscopic images of cilia activity were videotaped and CBF was later assessed in slow motion. Compared with baseline, group I showed no difference in CBF throughout the study. CBF was increased in group II from mean 9.7 (SD 0.4) to 11.2 (0.4) Hz (16%, P<0.001) and in group III from 9.5 (0.6) to 12.1 (0.5) Hz (28%, P<0.001) at 120 min. After 240 min of exposure to 95% oxygen, the CBF trend in group III was reduced to 11.8 (0.6) Hz but still remained above baseline. We conclude that oxygen appeared to have a dose- and time-dependent accelerating effect on CBF. Prolonged exposure to high oxygen concentrations reversed this trend. Direct oxygen toxicity (“oxygen stress”) is a possible explanation for this effect. These changes may result in impaired MTR. (Br. J. Anaesth. 1998; 80: 660–664)

Keywords: lung, trachea; lung, mucus; oxygen, toxicity

Subjects and methods

After obtaining approval from the Ethics Committee (Landesärztekammer) and informed subject consent, we studied 20 non-smoking volunteers. None had acute or chronic diseases of the upper respiratory tract or were receiving any medication that would affect the respiratory system. On three different occasions, they were scheduled for tissue sampling at the same time of day. Fresh samples of superficial mucosa of the inferior nasal turbinates were obtained using a fine curette. Samples were transferred immediately onto slides and wetted with nutrient medium (RPMI 1640 (10 Lsg), Dulbeccos, Eggenstein, Germany). To evaluate viability (visible ciliary beating), each sample was viewed in a hanging drop using a high resolution interference contrast microscope (Leitz, Orthoplan ICTL, Leitz, Wetzlar, Germany). The microscope table was heated to 37°C. A conventional air-conditioning system maintained the surrounding room air (21% oxygen) at 65–75% humidity. Viable tissue was loaded onto a polycarbonated membrane (Transwell-clear, 3.0 μm pore, TC treated, Costar Corporation, Cambridge, MA, USA). The tissue carrying membrane was mounted into a special exposure chamber, so that the base just dipped into the nutrient medium. We used a specially designed chamber (HWS 2×4,5l; H W Schmidt-Labortechnik, Mainz, Germany) to expose the tissue to various atmospheric environments (fig. 1). The volume of the chamber was 4.5 litre. Inside temperature and humidity were controlled by warm water at 37°C and 99% relative humidity, respectively (testoterm-601, Testo GmbH, Lenzkirch, Germany). The nutrient solution was buffered to pH 7.4 using 5% carbon dioxide added to the various atmospheric environments and repeat measurements were performed to monitor actual pH (pH-Meter 761 Calimatic, Knick, Elektrische Messgeräte, Berlin, Germany) and osmolarity (Advanced Microosmometer, Model 3M0 Plus, Advanced Instruments, MA, USA). Air and 5% carbon dioxide, and then various oxygen–carbon dioxide mixtures were passed through the chamber at a flow rate of 1.5 litre min⁻¹ controlled by a flowmeter (Rotameter,

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Drägerwerke, Lübeck, Germany). The oxygen concentration within the chamber was monitored by an oxygen sensor (Datex Capnomac II, Hoyer-Bremen, Germany). Thus the cells were wetted and supplied with nutrients by capillary forces through the pores of the membrane while the surface was exposed to a variety of oxygen concentrations.

Tissue was dissected into small pieces using a micropipette and moistened with RPMI 1640. After a primary stabilization period of 1 h in the exposure chamber (21% oxygen), samples were loaded onto coverslips, which were then placed upside down on a welled microscope slide. Using the same interference contrast microscope at high resolution (oil immersion at ×1200), the activity of the ciliated cells was viewed and a baseline recording obtained. The image from the microscope was transmitted to a high resolution video system (Videorecorder, Panasonic AG-6720, Matsushita Deutschland, Germany; Video-camera, Sony AVC-D1, Sony Deutschland, Köln, Germany) and recorded onto videotape. Tissue samples were then exposed to different oxygen concentrations over a period of 120 min. At 30, 60, 90 and 120 min of exposure, cilia activity was videotaped. For 50% of the cohort, exposure time was prolonged to 240 min.

To determine CBF, video-recordings were later viewed in slow motion and CBF of each sample and area were counted manually and averaged by an investigator blinded to the oxygen concentration. Subsequently, percentage change from baseline CBF was calculated at each time for each tissue.

The harvested cells of each individual were allocated randomly to one of three groups, thus ensuring that respiratory epithelial cells of each volunteer were exposed to all three gaseous environments. Tissue in group I (n=20) was exposed to a mixture of 21% oxygen and 5% carbon dioxide during the entire study and served as controls. The experimental groups received higher concentrations of normobaric oxygen: group II samples (n=20) were exposed to a room air–oxygen–carbon dioxide mixture (60% oxygen and 5% carbon dioxide) and group III samples (n=20) to a oxygen–carbon dioxide mixture (95% oxygen and 5% carbon dioxide), respectively.

**STATISTICAL ANALYSIS**

Statistical analysis was performed using Wilcoxon matched pair signed rank tests, assuming unequal differences. Significance was taken at $P<0.05$.

**Results**

No complications were noted while harvesting the tissues from the inferior nasal floor of the volunteers. Greater than 95% of the sample contained enough viable cells and were used in the study. Using
interference phase microscopy, we measured CBF in a total of 3600 cells. Under room air conditions, baseline mean CBF was 10.2 (SD 0.9) Hz (group I) (table 1). Group I served as the control and showed no difference in CBF throughout the study. After 240 min of exposure to room air, mean CBF was similar to that of baseline (10.4 (1.0) Hz). In contrast, exposure to increased concentrations of oxygen increased CBF in a dose- and time-dependent manner (table 1). The increase in CBF compared with controls (group I) appeared more rapid with the higher (95%) than with the intermediate (60%) oxygen concentration (fig. 3).

In group II, baseline mean CBF (9.7 (0.4) Hz) increased by approximately 5% to 10.2 (0.4) Hz after 30 min of exposure to 60% oxygen ($P<0.002$). With 95% oxygen (group III), the increase in CBF was greater, reaching maximum values of 9.5 (0.6) Hz at 2 h after 12.1 (0.5) Hz after 2 h of exposure (28% increase, $P<0.001$). When exposed for 4 h, the activity decreased to 11.8 (0.6) Hz (6% decrease from the maximum value at 2 h, $P<0.002$) (table 1).

**Discussion**

A large number of patients are dependent on continuous high inspiratory oxygen concentrations because of pulmonary disease. Many breathe spontaneously, while others require ventilation using various apparatus in intensive care units or operating rooms. However, the long-term influence of high normobaric oxygen on ciliary function of human respiratory epithelium has never been investigated. It is difficult to extrapolate results from animal studies on different species to human ciliary beat function and viable epithelial cells from humans are available only through invasive procedures such as bronchoscopy. However, Rutland and colleagues and Roth and colleagues described similar ciliary beat patterns of tracheal and nasal epithelia; thus, we decided to use nasal tissue samples from healthy subjects. The influence of different agents on respiratory epithelium has been studied by calculating CBF after exposure. Human respiratory epithelium is highly sensitive to dehydration, and changes in pH and temperature; thus an aqueous milieu was mandatory. We used a specially designed exposure chamber, established recently by Riechelmann and colleagues, to provide constant temperature, humidity, air flow and oxygen concentrations. The nutritive solution was maintained at 37.0°C and pH 7.5 and in controls (21% oxygen) there was no change in ciliary activity throughout the study. CBF was measured using the transmitted light technique combined with a high

<table>
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<tr>
<th>Time (min)</th>
<th>21% Oxygen</th>
<th>% Baseline</th>
<th>60% Oxygen</th>
<th>% Baseline</th>
<th>95% Oxygen</th>
<th>% Baseline</th>
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<tr>
<td>0</td>
<td>10.2 (0.9)</td>
<td>0.0</td>
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<td>9.5 (0.6)</td>
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<td>60</td>
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<td>0.6</td>
<td>10.7 (0.4)</td>
<td>10.8</td>
<td>11.6 (0.5)</td>
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<tr>
<td>90</td>
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<td>0.9</td>
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<td>11.9 (0.7)</td>
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<tr>
<td>240</td>
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<td>0.3</td>
<td>11.3 (0.3)</td>
<td>17.0</td>
<td>11.8 (0.6)</td>
<td>22.6</td>
</tr>
</tbody>
</table>

**Figure 3** Ciliary beat frequency (CBF), expressed as percentage change from baseline, in different gaseous environments (21%, 60% and 95% oxygen). **P<0.001** compared with groups II and III; †† P<0.01 compared with 120 min exposure to 95% oxygen (group III). (See results for more details.)
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speed video-microscope. In pilot experiments, this method was found to correlate well with the correlation analysis laser light scattering system (CALLS) techniques. We found basal ciliary beating of all harvested cells at a frequency of 10.2 (0.9) Hz. This was similar to the report of Jackson and colleagues, while Dulfano and colleagues reported basal CBF of respiratory epithelium at 12.8 Hz.

After exposing tissue samples for 2 h to different hyperoxic gas mixtures, we found a dose- and time-dependent increase in CBF of up to 28% with 95% oxygen compared with baseline. However, prolonged exposure (4 h) caused a 6% decrease in CBF compared with the high activity state after 2 h of exposure. These two important findings need to be discussed further in the context of the recent literature. First, the observed continuous increase in CBF within the first 2 h of exposure to high oxygen concentrations was similar to that of Harrison, Wong and Yeates in a canine model. Other groups found a significant increase in CBF after exposure to different mediators related to inflammation. Bonin, Phillips and McCaffrey and Khan, Dolata and Lindberg found that inflammatory substances (PGF2α, prostacyclins) can upregulate CBF in respiratory cells. Substance P seems to have similar effects. Rapid increases in CBF were also induced by “free radicals”. Thus our findings suggest a direct effect of oxygen on human respiratory cells.

Prolonged oxygen exposure may trigger a cascade of inflammatory mediators (PGF2α, prostacyclins, substance P) subsequently leading to inflammation of the respiratory mucosa and altered clearing of mucus. However, some toxic agents can cause an immediate reduction in CBF, even after brief exposure. Kukreja and colleagues reported inactivation of Ca2+-ATPase under H2O2 resulting in reduced cellular concentrations of ATP. Yoshitsugu and colleagues induced a decrease in CBF after application of HO2 for 5 min. These findings may be explained by a direct toxic effect of these substances on the epithelium, immediately impairing ciliary function. Additionally, they may relate ciliary activity to the energy status of respiratory cells.

The second important finding of our study was a delayed decrease (6%) in CBF after prolonged exposure (4 h), again confirming the findings of others. Boat investigated CBF of tracheal epithelium of newborns under different oxygen concentrations. He found ciliary stasis at 48–96 h after exposure to 80% oxygen. The observed reversibility of the early increase in CBF may represent progressive energy failure or direct oxygen toxicity at a cellular level after prolonged exposure to high oxygen concentrations. However, further studies are needed to clarify the exact mechanisms. Study design should allow tissue to recover from oxidative stress or provide additional ATP during prolonged exposure. Also, the exact time course of the decrease in CBF needs to be investigated over extended periods (i.e. 48–96 h). Further research in this area is important and clinically relevant, as prolonged exposure to normobaric hyperoxia reduces the mucociliary transport rate. Sackner and colleagues observed a 40% decrease in mucociliary transport rate after 2 h in dogs breathing a hyperoxic gaseous mixture. Additionally, the role of discoordination of ciliary beat as a result of cellular damage needs to be addressed. In inflamed respiratory epithelium, reduced mucociliary transport rate is a result primarily of discoordination with reduced CBF being less important.

In summary, our data suggest that oxygen had a dose- and time-dependent accelerating effect on CBF of human respiratory cells up to 2 h after exposure. In the context of recently published work, this may be explained by “oxygen stress” to the respiratory epithelium at a cellular level. In addition, we were able to show that during prolonged exposure (>2 h), high oxygen concentrations (95%) reduced CBF. Both an increase and late reduction in high CBF represent patterns of impaired physiology of human respiratory ciliary cells and may indicate impaired mucociliary transport capacity of these cells. From a clinical perspective, we conclude that prolonged exposure to high oxygen concentrations appears to indicate increased risk of respiratory infection and therefore should be avoided whenever possible.

References


