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An in vitro model to study effects of airborne pollutants on human ciliary activity*

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SUMMARY

A method to study the effects of airborne pollutants on ciliary activity of isolated human respiratory cells is presented. Human respiratory cells were scraped from nasal cavities of 25 healthy volunteers and washed in Ringer's solution. The cells were placed on polycarbonate membranes (3 μm pore size) and kept in macroplate holders swimming on Ringer's solution. Cells were thus kept humid and were supplied with nutrients through the pores of the membrane by capillary forces, while their surface was exposed to the gaseous environment. Isolated respiratory cells were exposed to SO_2, NO_2, and mixtures of SO_2 and NO_2 in various concentrations for 30 min and for 2 h. Exposure to synthetic air served as control. Ciliary beat frequency was measured using video-interference contrast microscopy, before and after exposure to the various gases. Exposure of isolated respiratory cells to a non-toxic gaseous environment resulted in a 20% reduction of ciliary beat frequency. A concentration-dependent decrease of ciliary beat frequency following exposure to SO_2 in concentrations ranging between 2.5 and 12.5 ppm was found. Exposure to NO_2 up to 2 h in concentrations ranging between 3 ppm and 15 ppm did not decrease ciliary beat frequency. No cumulative effect was found, if a mixture of SO_2 (2.5 ppm) and NO_2 (12 ppm) was applied.

Key words: airborne pollutants, ciliary activity, toxicity, human

INTRODUCTION

The number of chemicals, including numerous volatile substances, that are potentially hazardous to human airways has increased rapidly. Responses of human airways to potentially hazardous agents are generally predicted from experiments done in other species, mainly rodents. Extrapolating results in laboratory animals to human airways is problematic, since various differences between animal and human airways have been reported (Brain, 1988).

An *in vitro* model to investigate acute effects of volatile substances on ciliary activity of human respiratory cells is presented. The basic principle is to expose isolated human respiratory cells to a toxic atmosphere. Using various concentrations of SO_2 and NO_2 the reproducibility and concentration-response performance in this experimental set-up was evaluated.

METHODS

Human respiratory cells were obtained from 25 healthy non-smokers (mean age 24±4 years; 11 females, 14 males). Using a fine curette, a superficial sample from the mucosa of the posterior two-thirds of the nasal floor was harvested and suspended in Ringer's solution (pH 7.4) at a temperature of 37°C. Subsequently, the sample was placed in a small container. The bottom of this container consisted of a polycarbonate membrane with a pore size of 3 μm (Nucleopore; Tübingen, Germany). The container was placed into tissue-culture plates (NUNC; Denmark). The plates were filled with pre-warmed Ringer's solution, until the fluid level just reached the bottom surface of the polycarbonate membrane. Thus, the cells were humidified and supplied with nutrients by capillary forces through the pores of the membrane, while their surface could be exposed to various gaseous environments (Figure 1).

The preparations were exposed for 30 and 120 min to analytical grade spirometry-calibration air (control: 5% CO_2, 15.9% O_2 and 79.1% N_2. Linde; Unterschleissheim, Germany), to the same spirometry air with SO_2 in concentrations ranging between 2.5 ppm and 12.5 ppm, to spirometry air with NO_2 in concentrations ranging between 3 and 15 ppm, and to spirometry air with a mixture of SO_2 (2.5 ppm) and NO_2 (12 ppm), in a standard exposure system at 37°C and 98-100% humidity (Figure 2). Class-I calibration gases (Linde; Unterschleissheim, Germany)

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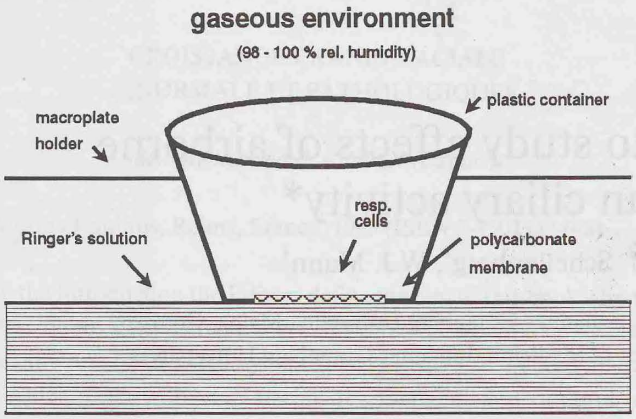

Figure 1. Gas exposure of isolated human respiratory cells. Cells obtained by nasal scrapings were washed in Ringer's solution, placed on a polycarbonate membrane and kept swimming on Ringer's solution. Cells were kept moist through the pores of the polycarbonate membrane by capillary forces.

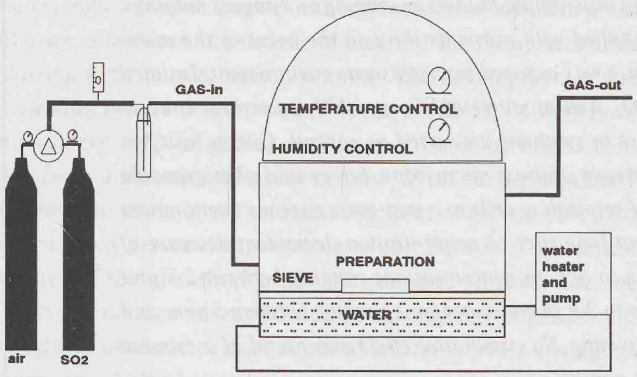

Figure 2. Gas exposure system. Synthetic air and SO_2 or NO_2 were mixed in concentrations desired. Gas flow was 1.5 l/min. Preparations were placed on a sieve above a water bath, which kept temperature and humidity inside the chamber constant (37°C and 98–100%, respectively).

were mixed with the spirometry air using a high-precision gasfusion unit (accuracy > 99.5%. Linde; Unterschleissheim, Germany). Gas flow was set to 1.5 l/min. Accuracy of gas concentrations was semi-quantitatively checked using Draeger detection tubes (Draegerwerk; Lübeck, Germany). Reversibility of toxic effects of SO_2 was evaluated suspending the cells in phosphate-buffered solution following gas exposure.

To measure ciliary activity, a small part of each sample was placed on a coverslip. The coverslip was placed upside down on a welled microscopic slide and ciliated cells were visualized using an interference contrast microscope (Leitz Orthoplan; Wetzlar, Germany) with a heatable microstate (37°C). Recordings of ciliary beat frequency and beating pattern of the ciliated cells were made with a video-recorder connected to the microscope via a CCD-videocamera and evaluated in slow motion. Ciliary beat frequency of respiratory cells in 5 high-power fields per sample (oil immersion; ×1,200) was counted and averaged.

To assess the effects of exposure to synthetic air alone, ciliary beat frequency before and after exposure was compared. Evaluating the effects of SO_2 and NO_2, ciliary beat frequency after exposure to the toxic gases was compared with ciliary beat frequency after exposure to synthetic air (control). Significance of results was tested using the Kruskall-Wallis one-way analysis of variance.

RESULTS

It was found feasible to expose isolated human respiratory cells to a gaseous environment maintaining their cellular function. Ciliary beat frequency of isolated respiratory cells was reduced from 10.4±0.9 Hz directly following harvest to 7.8±0.8 (n=30) following 30-min exposure to synthetic air with 5% CO_2 and to 8.6±1.1 Hz (n=9) following 120-min exposure, respectively. Thus, ciliary beat frequency of isolated respiratory cells decreased approximately 20–25% (Figure 3) due to exposure to the non-toxic gaseous environment over a period of 30–120 min.

Ciliary beat frequency revealed a concentration-dependent reduction ($p < 0.001$) following exposure to SO_2 (n=14) from 7.8±0.8 Hz (control) to 4.7±1.7 Hz (2.5 ppm), 2.8±1.4 Hz (5 ppm), 1.5±1.4 Hz (7.5 ppm), 0.7±1.2 Hz (10 ppm), and 0.28±0.75 Hz (12.5 ppm), respectively (Figure 4, left side). The

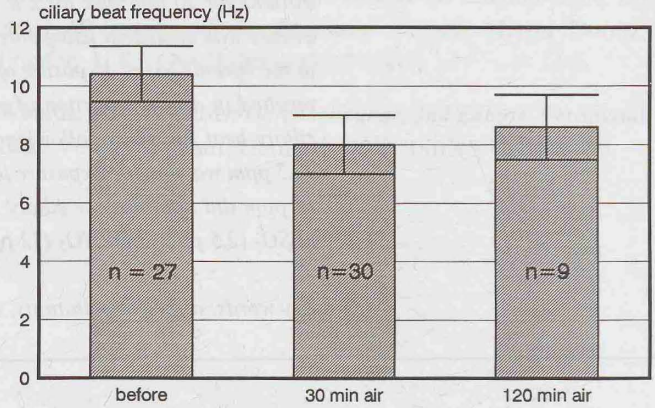

Figure 3. Ciliary beat frequency of human ciliated nasal cells directly following harvest and following exposure to synthetic air containing 5% CO_2 for 30 and 120 min, respectively (mean ± SD).

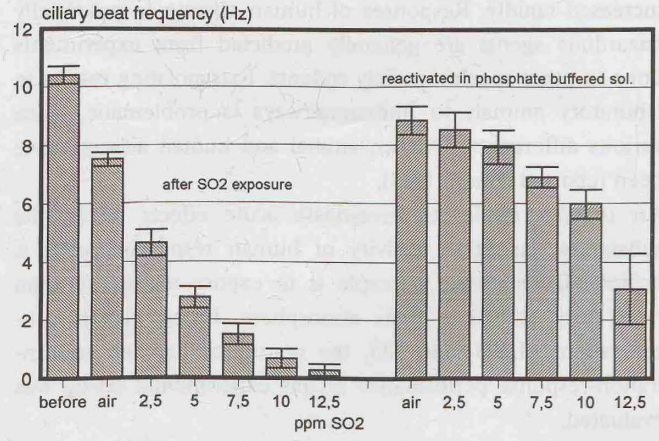

Figure 4. Concentration related decrease ($p < 0.001$) of ciliary beat frequency (mean±SEM; n=14) of isolated human respiratory cells to SO_2 (left bars). If samples were suspended in phosphate buffer following SO_2 exposure, the decrease of ciliary beat frequency was in part reversible (right bars; n=8). "Before": directly following harvest; "air": 30-min control exposure to synthetic air containing 5% CO_2.

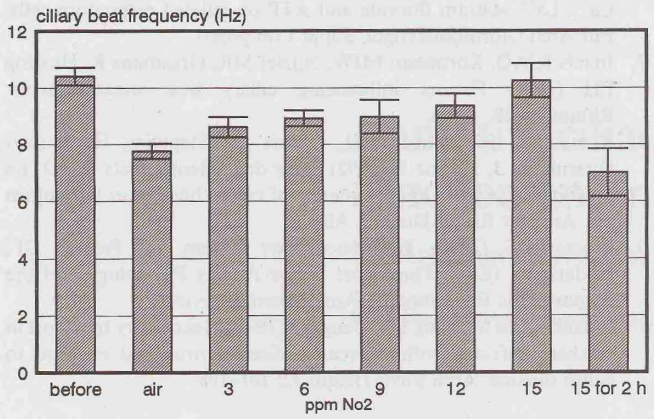

Figure 5. Concentration-related increase ($p < 0.025$) of ciliary beat frequency (mean±SEM; n=9) of isolated human respiratory cells following exposure to NO_2 for 30 min and decrease following exposure to 15 ppm NO_2 for 120 min. "Before": directly following harvest; "air": 30-min control exposure to synthetic air containing 5% CO_2.

effects of SO_2 were in part reversible, when the samples were suspended in phosphate-buffered solution following SO_2 exposure (n = 8; Figure 4, right side).

If ciliated cells were exposed for 30 min to NO_2, ciliary beat frequency significantly ($p < 0.024$) increased from 7.8±0.8 Hz (control) to 8.6±1.0 Hz (3 ppm), 8.9±0.8 Hz (6 ppm), 9.0±1.6 Hz (9 ppm), 9.4±1.2 Hz (12 ppm), and 10.3±1.6 Hz (15 ppm), respectively (n=9; Figure 5). Exposure to 15 ppm NO_2 for 120 min (n=9) resulted in a non-significant decrease of ciliary beat frequency to 7.0±0.84 Hz.

Exposure to mixed SO_2 (2.5 ppm) and NO_2 (12 ppm) resulted in a reduction of ciliary beat frequency from 7.8±0.8 Hz (control) to 4.33 Hz (n=8), comparable to the effect of 2.5 ppm SO_2 alone.

DISCUSSION

Facing an increasing number of volatile substances, cost-effective and time-saving methods are required to assess potentially hazardous effects on human mucociliary transport. To study the ciliary beat frequency of respiratory cells is one method to examine the propelling force of the mucociliary transport system. Extrapolation of results in animal studies to human mucociliary transport is difficult (Brain, 1988) and effects of volatile substances on human ciliary activity could not yet be accurately measured in an experimental model. Thus, only a few volatile chemicals have been investigated using human exposition models regarding their effect on mucociliary transport.

In previous investigations, it had been proven valuable to study the effects of various agents on ciliary activity in scrapings or brushings of human respiratory cells (Braga, 1989; Ingels et al., 1991; Hafner et al., 1993). Since human respiratory cells are remarkably sensitive to dehydration (Proctor, 1982), an aqueous milieu was mandatory. The experimental set-up used in this study allows to expose isolated human respiratory cells to a water-saturated gaseous environment, keeping cells humid by capillary forces through the pores of a polycarbonate membrane.

Comparing ciliary beat frequency before and after exposure to synthetic air for 30-120 min, a reduction of approximately 20–25% was consistently found. This reduction of ciliary beat frequency resembles that of ciliated cells suspended in Ringer's solution and might be due to cellular ATP consumption and Ca^{2+} loss (Hafner et al., 1993). In addition, mechanical damage of cells during harvest and slight dehydration during air exposure might contribute to this reduction. However, the variation of ciliary beat frequency before and after exposure to synthetic air remained almost unchanged. The standard deviation of ciliary beat frequency was 8.7% of the mean directly following harvest, and 10.2% of the mean following 30 min exposure to synthetic air. This suggests that the effects of air exposure (control) on ciliary beat frequency are uniform and predictable. Thus, the exposure model introduced in this study allows to separate the effects of mere exposure of ciliated cells to an innocuous gaseous environment from possible noxious effects of airborne pollutants.

Comparing the effects of SO_2 exposure in concentrations ranging between 2.5 and 12.5 ppm with exposure to synthetic air (control), a concentration-dependent decrease of ciliary beat frequency was found. Almost identical effects of the same concentrations of SO_2 had been observed in tracheal specimens of guinea pigs (Kienast et al., 1992). The effects of SO_2 on ciliary activity were in part reversible, when the cells were suspended in phosphate-buffered solution following gas exposure. This suggests that the effect of SO_2 on ciliary activity are in part due to pH-related cellular dysfunction.

The effects of SO_2 on the mucociliary system observed in these experiments are consistent with results obtained in *in vivo* studies. In newborn chicken, Wakabayshi et al. (1977) found a significant decrease of mucus transport velocity at 1.4 ppm SO_2. In rabbits, exposure to 10 ppm SO_2 for 10 min resulted in complete cessation of ciliary activity (Dalhamn and Strandberg, 1961). In human volunteers, no significant reduction of nasal mucus flow was observed at 1 ppm SO_2, but a significant decrease at 5 ppm SO_2 (Andersen et al., 1974).

Exposure of isolated respiratory cells to NO_2 ranging between 3 and 15 ppm for 30 min resulted in a significant, concentration-dependent increase of ciliary beat frequency from 7.8 ± 0.8 Hz to 10.25 ± 1.57 Hz. This increase was of short duration, since exposure to 15 ppm NO_2 for 120 min resulted in a non-significant decrease of ciliary beat frequency. The underlying mechanisms are unclear, since reflex-mediated ciliary acceleration as reported for low concentrations of irritant gases applied in vivo can be ruled out. Since NO_2 dissolves to HNO_3, a strong oxidant, the shortly increased ciliary activity may be related to oxidation of cell membrane molecules. This could induce an initial cellular stress reaction, that precedes cellular dysfunction (Amdur, 1991).

In conclusion, gas exposure of isolated human respiratory cells is a cost-effective and time-saving method to evaluate possible risks of volatile substances for ciliated respiratory cells. This method offers interesting perspectives in detecting occupational risk factors for the respiratory tract while working with volatile substances. Exposure to 2.5 ppm SO_2 for 30 min resulted in a

significant decrease of ciliary activity. Exposure to 15 ppm NO_2 for 30 min did not reduce ciliary activty, whereas exposure to 15 ppm NO_2 for 120 min resulted in an insignificant ciliary inhibition. No cumulative effect was found if combined SO_2 (2.5 ppm) and NO_2 (12 ppm) were applied.

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