Topical Reviews

The assessment of nasal mucociliary clearance and the effect of drugs

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Introduction

Mucociliary clearance provides an important protective function in the nose. A variety of topically and systemically administered drugs are used to treat nasal pathologies and there is evidence that these therapies may modify nasal mucociliary clearance (NMCC). This review describes the methods of assessing NMCC and the effects upon it of therapeutic agents.

Overall, mucociliary clearance is dependent on an adequate quantity of mucus of appropriate viscosity and elasticity, and on adequately functioning cilia. The nasal mucus blanket consists of two layers. The outer mucus layer (gel phase) is relatively viscous and moves over the surface of the cilia, which are surrounded by a serous periciliary fluid layer (sol phase) (1). The ciliated cells have between 120 and 200 cilia that project from the luminal surface of the cell into the mucus. In the anterior part of the nose, there is a paucity of cilia and the mucus moves slowly at about 1–2 mm h⁻¹. In the posterior part of the nose, mucus moves more rapidly at 10 mm min⁻¹. The beat of cilia is quick and forceful in a forward propulsive stroke that is followed by a slower recovery stroke, and beat frequency in man is about 13 Hz in vitro at 37°C. Beating activity is coordinated so that it appears to move from one region to another adjacent posterior one in a metachronous fashion. Fine particulate matter, including bacteria, that enters with inspired air is filtered by adherence to the mucus film. This occurs both by impaction and as the result of an electrostatic surface charge. Most particles, including microorganisms, trapped by this moving carpet of mucus in the nose are propelled towards the pharynx where they are swallowed (2). A defect in any part of this system such as occurs in primary or secondary ciliary disorders or cystic fibrosis, predisposes the individual to infection of the nose and paranasal sinuses (3).

Measurement of mucociliary clearance in vivo

Several techniques have been developed to measure nasal mucociliary clearance in vivo and these have the advantage of being suitable for assessment of prolonged treatment effects. Wanner (4) concluded that mucociliary activity could be assessed by two basically different methods. Soluble tracers such as saccharin dissolve in the gel and sol phases of the mucus and so measure transport of both sol and gel layers. Marker particles placed on the mucosa measure transport of the outer mucus layer. In normal conditions, both layers probably move simultaneously in the direction of the beating cilia and this is supported by the correlation observed between particle and saccharin transport times (5). There is evidence however, that in some circumstances the periciliary fluid layer can be propelled more rapidly than the overlying mucus layer (5–7) so that movement of the two layers may be dissociated.

Saccharin test

The most widely used method for in vivo assessment of NMCC is the saccharin test (8,9). This is commonly performed by first asking the subject to blow the nose to remove any excess secretions. A saccharin particle is placed on the medial surface of the inferior turbinate of one nasal cavity, at least 7 mm behind the anterior end to avoid the area of mucosa where cilia beat in an anterior direction. The time from particle placement until the subject reports the first sensation of a sweet taste is measured and expressed as a clearance time. As a screening test to detect abnormal mucociliary clearance, the saccharin...
test is reproducible (10,11), inexpensive, readily available and simple to perform in large numbers of patients (10). It has been recommended that those patients with clearance times of greater than 60 min should be investigated further for abnormalities of either mucus or ciliary components of clearance (10).

DYE METHOD

Other methods have utilized a dye to give a visual representation of nasal clearance based on the time taken for the dye to appear in the nasopharynx following its application to the anterior nasal cavity. In a study using indigo carmine, clearance times were longer than those obtained using saccharin, but the measures were positively correlated (12). Other coloured substances used to evaluate nasal mucociliary transport have included charcoal powder (13) and edicol-orange (14).

RADIOGRAPHIC METHOD

Nasal mucus velocity has been estimated by the use of 1 mm discs of Teflon, rendered radiopaque by bismuth trioxide. Five to ten of these are placed on the superior surface of the inferior turbinate and a fluoroscopic image intensifier used to follow their motion (15). In some cases one or more discs do not move, as a consequence of variability in pathways of nasal flow, indicating a shortcoming in methods of assessing mucus velocity which use only one particle. Employing this method, group mean nasal velocities in healthy subjects have been recorded in the range 6.8-10.8 mm min$^{-1}$. Values of nasal mucus velocity do not necessarily correlate with saccharin clearance times. The lack of correlation may be attributable in part to the solubility of saccharin in both gel and sol layers of mucus which contrasts with the insoluble nature of the Teflon discs. This method has the disadvantage of exposure to X-rays and is of greater complexity than the saccharin test.

RADIOISOTOPIC METHOD

Radioisotopes have also been used and their passage along the nasal cavity recorded by the use of a collimator and scintillation counter placed alongside the subject’s face. These techniques have employed droplets of radiolabelled solutions, for instance $[^{99m}Tc]$ phytate (16), or saline labelled with $[^{99m}Tc]$ pertechnetate (19). Although the use of an insoluble particle overcomes the problems of dispersion and streaming, single particles have been observed to remain stationary (19). Furthermore, methods employing insoluble radiolabelled particles are regarded (7) as assessing movement of the outer mucus layer, in contrast to those methods which employ soluble tracers and measure transport of both sol and gel layers. Radioisotope techniques have the advantage of permitting visual record of the progress of the labelled agent and determination of transit rate in different parts of the nasal cavity, and it has been noted that transit rate tends to increase in the posterior portion of the nasal cavity (19). Furthermore, they are more likely to reflect mean clearance than the saccharin method which measures clearance time of the most rapidly moving particles. However, they need expensive equipment to perform and little assessment has been made of their reproducibility.

Techniques for sampling human nasal epithelial cells

Both human and animal studies have suggested that cilia play an important role in maintaining the functional integrity of the airways and that efficient mucus clearance is dependent on coordinated patterns of ciliary activity and the total numbers of cilia involved. Investigations of nasal ciliary activity in vitro have consequently focused on measurements of ciliary beat frequency (CBF) in nasal epithelial tissue obtained in one of several ways:

NASAL BRUSHING

Nasal brushing is a non-invasive technique, which is relatively simple to perform, and may be carried out either in the absence or presence of local anaesthesia. It is suitable for use in young children and patient tolerance is good. Generally a nylon brush of about 2 mm diameter is used to harvest the nasal mucosa from (i) the lateral nasal wall (20), (ii) between the turbinate and the lateral wall of the nasal cavity (21), (iii) the nasal septum at the level of inferior turbinate (22) and (iv) the inferior nasal turbinate itself (23), for direct observation. Multiple samples can be obtained at one time and brushing can be repeated. Using this sampling technique it is also possible to obtain single ciliated cells and strips
of ciliated epithelium, which are suitable for ultrastructural studies (21).

A major disadvantage of the technique is that the area being sampled is small and therefore may not be representative of the actual site where any pathogenic features are being expressed. Also since all samples contain cells which are beating at different frequencies, it is suggested that small groups of ciliated cells are not used and that only cells which are fixed in tissue are investigated for CBF counts (23).

CURETTE AND FORCEPS BIOPSIES

Both these techniques are relatively simple and can be easily executed by trained personnel. The procedures are carried out under local anaesthesia with minimal discomfort to the patient and allow sampling of the nasal mucosa under both natural and experimental situations. Both techniques have been widely used in obtaining human nasal epithelial cells (24,25) and it has been suggested that the number and the vitality of cells obtained is dependent on the sharpness of the curette and biopsy forceps and the site and force used for sampling.

Ingels and colleagues (26) have compared the two sampling methods and concluded that forceps biopsies are preferable to curette obtained specimens, since the beating pattern and CBF in samples taken by biopsy forceps was more constant than in curette samples. These authors have also suggested that a 'continuous layer of ciliated cells', in contact with basal membrane is desirable for measurements of CBF in vitro.

NASAL SURGERY

The major limitation of the techniques described above is the small quantity of specimens obtained. In contrast, nasal surgery, e.g. turbinectomy and polypectomy provide abundant material for both direct observation (27) and for culture of nasal epithelial cells which have also been used in investigations of nasal ciliary activity (28).

Measurement of ciliary beat frequency in vitro

Since the early 1930s (29), several attempts have been made to study ciliary motility. In both human and mammalian epithelium, the cilia beat at such high frequencies that direct observation allows only the registration of dramatic changes in the CBF, and therefore makes this form of investigation of limited value, except in the case of total arrest of ciliary motility (30). Although more recent investigations involving measurement of ciliary activity in vitro, have employed more sophisticated techniques, these too have had inherent problems. Additionally, due to variability in values of CBF in samples obtained by either brushing, curette or biopsy forceps, different investigators have set different criteria for the type of cells to study. For example Deitmer and colleagues (31) and Bernstein and colleagues (32) have suggested that the ciliary activity of only the most 'actively beating cells' should be investigated, since only these cells represent the majority of ciliated cells in the epithelial tissue under investigation. Devalia and colleagues, on the other hand, have taken into consideration the variability in the ciliary activity in different areas of the airway tissue and measured the CBF in randomly chosen areas of cultured human bronchial and nasal epithelial cells (28). More recently, Rusznak and colleagues have demonstrated that the ciliary beat frequency of cultured human bronchial epithelial cells, in vitro, may be influenced by diurnal rhythms (33). These authors suggest that in studies involving repeated measurements of ciliary activity in human airway tissue, over a period of several days, measurements of ciliary activity and associated parameters such as changes in intracellular concentrations of cAMP and Ca" should be made at the same time each day.

VISUAL METHOD

Forrest and colleagues (34) employed a simple way of assessing ciliary motility. Nasal mucosa from the inferior turbinate was examined directly after biopsy, by phase contrast light microscopy, and the ciliary motility was assessed on a graded scale of 0-3; 0 being absent, 3 being vigorous. Stafanger and colleagues (25) have investigated, by direct visualization, the degree of irregularity in ciliary beat, graded from 0 (complete synchrony) to 5 (complete asynchrony), and suggested that the pattern of ciliary beat is of significance, since the synchrony in ciliary beat determines the effectiveness by which the airways remove particles entrapped in the mucus. Despite the simplicity and the relative low cost of the direct observation technique, this method has serious limitations with regard to accuracy, owing to the investigators's subjectivity.

AUDIO METHOD

Bleeker and Hoeksema (35) have used an auditory clicking device and made measurements of ciliary beat by synchronizing the click with the visually observed ciliary action. This method is highly subjective and prone to gross errors in its final assessment.
STROBOSCOPIC METHOD

Essentially this method involves synchronization of a stroboscope to the ciliary beat frequency viewed under a microscope (36,37). The flickering frequency of a stroboscope lamp is adjusted to the flickering of the light reflexes on the ciliated epithelium and at the point where the stroboscope frequency equals the ciliary frequency the cilia exhibit the least movement. This method produces a single frequency value for regions of a ciliary membrane but is unable to provide frequency time variations within individual cells or differences in frequencies between adjacent cells. Furthermore, although the principle of the method is simple, the device is difficult to use and inaccurate at very low values of CBF. Additionally, as the flicker of the epithelium may vary, large experimental errors may be introduced into the frequency determination (38,39).

CINEMATOGRAPHIC (PHOTOGRAPHIC) METHOD

This method was first introduced by Proetz who used motion picture camera to record ciliary motion (29). This involved the illumination of ciliated cells and recording of the reflected flickering light reflex on motion picture film by use of a high speed camera. Following development, the film was projected onto a screen at reduced speed and the frequency of ciliary beat visualized and counted directly off the screen (40). This technique was further developed by Dalhamn (41), and Sanderson and Sleigh (42) who, respectively, used cameras capable of recording at rates of 220 and 500 frames s⁻¹, thus allowing the estimation of the beat frequency with greater accuracy.

With the introduction of video techniques, the classical motion picture cameras have been replaced by sophisticated and easier-to-use video cameras (43), capable of high speed recording and slow motion and still picture replay, allowing the study of individual aspects of single beat sequence, including amplitude, beat direction and coordination (44).

PHOTOELECTRIC METHOD

Photoelectric technique, in its various forms, is the most widely used technique for measuring CBF (45–47). In principle the technique allows a photosensitive cell to detect light variations produced by the movement of cilia, utilizing either transmitted (40,48) or reflected light (35). These techniques require the use of a parallel light beam which illuminates the epithelial surface along the visual axis. The varying reflection of the light from the cilia causes voltage variations in a photoelectric measuring device (photosensitive cell) connected to a photomultiplier and an amplifier. The frequency of the voltage variations, correspond to the rate of ciliary beat and is either counted electronically or recorded on an oscilloscope. Estimation of the CBF from the recordings on the oscilloscope can be performed by either counting the ‘blips’ on the oscilloscope screen (‘hand counting technique’) (49) or by a microcomputer performing the fast Fourier transform (digital spectrum analyser) which provides rapid and complete analysis of CBF (50). The mucus layer, the cell bathing medium and the irregularity of the reflecting cell surface, however, can contribute to the differences in the reflected light, making it difficult to know the exact origin of the surface reflections (51).

Teichtal and colleagues (52) have modified the transmitted light technique, by viewing the ciliated cells on a video display unit, and estimating the ciliary activity in the specimen by means of a hand held probe placed against the television screen in front of each region of beating cilia. This process allows representative groups of cilia to be investigated and according to the authors, CBF of only four to five cilia from one cell at a time can be detected.

The various transmitted light techniques appear to give reproducible results, are convenient to use and require minimal subjective assessment. Overall, the potential disadvantages of these techniques are detection of unrelated vibrations and the inability of some systems to detect light changes produced by a single cilium or even cilia from one cell.

LASER LIGHT-SCATTERING SPECTROSCOPY METHOD

The laser light-scattering spectroscopy technique employs a safe low power laser source to emit a fine laser beam, of approximately 15 µm diameter, directly onto the specimen and analyses the reflected laser light spectrum for fluctuations in the back-scattered light intensity resulting from the moving cilia. The fluctuations of the back-scattered light are detected by a photomultiplier tube and the spectral structure of the resulting photocurrents is assessed either by standard spectrum analysis or autocorrelation techniques. The method is simple, accurate, and reproducible, due to both the precise frequency and coherency of the laser light. The major disadvantages of the technique are that it is expensive and as is the case for all techniques measuring reflected light, is subject to error due to light scattered as a result of the slightest vibration in the fluid bathing the specimen (53).

Using this technique, Lee and Verdugo (54) have estimated that the activity of 200–300 cilia, corresponding to a surface area of 1–2 ciliated cells, can be measured readily.
ANALOGUE CONTRAST ENHANCEMENT TECHNIQUE
The analogue contrast enhancement technique takes into account several difficulties encountered by other techniques employed for the measurement of CBF, in vitro. Devalia and colleagues (28,55–57) have demonstrated that this is a highly reproducible and comparatively safe and simple technique, which can be readily performed by un-skilled personnel with minimal training. The technique involves transfer of the microscopic image of the specimen onto a television monitor, via a video camera, followed by electronic ‘stretching’ of the video signal such that the contrast is dramatically increased to allow visualization of the specimen far beneath the resolution limits of the optical microscope. Television signals relating to differences in light intensity resulting from ciliary motion at any specific point on the monitor screen, as pre-determined by positioning an electronic mouse-operated cross-hair light sensing probe directly over ciliated cells, are analysed by an on-line micro-computer, incorporating a PCX Video Digitiser Card and specifically programmed for this application. The differences in light intensities are computed directly into units of Hertz and therefore are not subject to any further manual or mathematical transformations.

The ciliary motion is measured in randomly chosen areas of the specimen, the positions of which can be noted accurately with a horizontal and vertical Vernier scale on the microscope stage such that it is possible to make further recordings of the ciliary motion in the same area, at different time points during the study period. The microscopic images of the ciliary motion are recorded by use of a video camera and a high resolution videorecorder, thus enabling an independent observer to analyse the CBF of the specimen in a blinded manner and at any time in the future, by playing back the recording (Fig. 1).

The effect of drugs on human nasal mucociliary clearance
A variety of topically administered drugs are used in the treatment of nasal disease, and the intranasal route may also be used to deliver to the systemic circulation compounds difficult to deliver by the oral route. These include pharmacologically active polypeptides and proteins which are currently being developed, such as hormones and vaccines. The consequences of inhibited mucociliary clearance suggest that constituents of preparations intended for nasal delivery should not adversely affect the mucociliary clearance system.

Nasal preparations of corticosteroids are widely used in the treatment of nasal disorders, particularly allergies, and most studies of their short term administration have shown little effect on NMCC in vivo. Thus, topical budesonide aerosol administered for 1 week to healthy volunteers (58) had no effect on nasal mucociliary clearance as assessed by the saccharin-dye test, and a further study of topical budesonide administered for 1 week in healthy subjects similarly found no reduction in clearance when post-treatment values were compared with baseline (59).
Table 1  The effect of drugs not exhibiting anti-allergic properties, on the ciliary beat frequency of human nasal epithelial cells, *in vitro*

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Concentration</th>
<th>CBF (% change)</th>
<th>Time</th>
<th>Reversibility</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anaesthetics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lignocaine hydrochloride</td>
<td>$4 \times 10^{-3} \text{M}$</td>
<td>NE</td>
<td>30 min</td>
<td></td>
<td>Rutland <em>et al.</em> (21)</td>
</tr>
<tr>
<td></td>
<td>$7 \times 10^{-2} \text{M}$</td>
<td>↓ (100%)</td>
<td>30 min</td>
<td></td>
<td>Rutland <em>et al.</em> (21)</td>
</tr>
<tr>
<td></td>
<td>$1.25 \times 10^{-3} \text{g ml}^{-1}$</td>
<td>NE</td>
<td>30 min</td>
<td></td>
<td>Rutland <em>et al.</em> (45)</td>
</tr>
<tr>
<td></td>
<td>$2.5 \times 10^{-3} \text{g ml}^{-1}$</td>
<td>↓ (4%)</td>
<td>30 min</td>
<td></td>
<td>Rutland <em>et al.</em> (45)</td>
</tr>
<tr>
<td></td>
<td>$2.0 \times 10^{-2} \text{g ml}^{-1}$</td>
<td>↓ (100%)</td>
<td>30 min</td>
<td></td>
<td>Rutland <em>et al.</em> (45)</td>
</tr>
<tr>
<td>cocaine hydrochloride</td>
<td>0.5%</td>
<td>↓ (100%)</td>
<td>90 min</td>
<td></td>
<td>Robson <em>et al.</em> (73)</td>
</tr>
<tr>
<td></td>
<td>1%</td>
<td>↓ (100%)</td>
<td>90 min</td>
<td></td>
<td>Robson <em>et al.</em> (73)</td>
</tr>
<tr>
<td>Morphine/morphinomimetics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>morphine</td>
<td>2 mg ml$^{-1}$</td>
<td>↓ (14%)</td>
<td>60 min</td>
<td></td>
<td>Hermens <em>et al.</em> (86)</td>
</tr>
<tr>
<td>fentanyl</td>
<td>10 μg ml$^{-1}$</td>
<td>↓ (10%)</td>
<td>60 min</td>
<td></td>
<td>Hermens <em>et al.</em> (86)</td>
</tr>
<tr>
<td>sufentanil</td>
<td>10 μg ml$^{-1}$</td>
<td>↓ (11%)</td>
<td>60 min</td>
<td></td>
<td>Hermens <em>et al.</em> (86)</td>
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<td>Antimicrobial agents</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>neomycin</td>
<td>5 mg ml$^{-1}$</td>
<td>↓ (82%)</td>
<td>6 h</td>
<td></td>
<td>Stanley <em>et al.</em> (58)</td>
</tr>
<tr>
<td>Antiviral agents</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ribavirin</td>
<td>500 μg ml$^{-1}$</td>
<td>↓-healthy</td>
<td>day 4</td>
<td></td>
<td>Dolovich <em>et al.</em> (22)</td>
</tr>
<tr>
<td></td>
<td>20 mg ml$^{-1}$</td>
<td>NE-rhinorrhoea</td>
<td>day 4</td>
<td></td>
<td>Dolovich <em>et al.</em> (22)</td>
</tr>
<tr>
<td></td>
<td>50 mg ml$^{-1}$</td>
<td>↓ (33%)</td>
<td>5 h</td>
<td></td>
<td>Han <em>et al.</em> (23)</td>
</tr>
<tr>
<td></td>
<td>60 mg ml$^{-1}$</td>
<td>↓ (47%)</td>
<td>5 h</td>
<td></td>
<td>Han <em>et al.</em> (23)</td>
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<td>α-agonists</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>naphazoline nitrate</td>
<td>0.1%</td>
<td>↓ (33%)</td>
<td>20 min</td>
<td>Yes</td>
<td>Van de Donk <em>et al.</em> (70)</td>
</tr>
<tr>
<td></td>
<td>0.1%</td>
<td>↓ (69%)</td>
<td>10 min</td>
<td>Yes</td>
<td>Curtis and Carson (76)</td>
</tr>
<tr>
<td></td>
<td>0.1%</td>
<td>↑ (15%)</td>
<td>3 h</td>
<td></td>
<td>Robson <em>et al.</em> (73)</td>
</tr>
<tr>
<td>phenylephrine hydrochloride</td>
<td>0.1%</td>
<td>↓ (46%)</td>
<td>3 h</td>
<td></td>
<td>Robson <em>et al.</em> (73)</td>
</tr>
<tr>
<td>a-antagonists</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>phentolamine mesylate</td>
<td>0.01 mg ml$^{-1}$</td>
<td>↓ (46%)</td>
<td>3 h</td>
<td></td>
<td>Robson <em>et al.</em> (73)</td>
</tr>
<tr>
<td></td>
<td>0.1 mg ml$^{-1}$</td>
<td>↓ (100%)</td>
<td>90 min</td>
<td></td>
<td>Robson <em>et al.</em> (73)</td>
</tr>
<tr>
<td>β$_2$ agonists</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>salbutamol sulphate</td>
<td>0.01 mg ml$^{-1}$</td>
<td>↑ (7%)</td>
<td>3 h</td>
<td></td>
<td>Robson <em>et al.</em> (73)</td>
</tr>
<tr>
<td></td>
<td>0.1 mg ml$^{-1}$</td>
<td>↑ (13%)</td>
<td>3 h</td>
<td></td>
<td>Robson <em>et al.</em> (73)</td>
</tr>
<tr>
<td>Terbutaline</td>
<td>$10^{-10} \text{M}$</td>
<td>↑ (17%)</td>
<td>60- min</td>
<td>Yes</td>
<td>Staskowski <em>et al.</em> (74)</td>
</tr>
<tr>
<td></td>
<td>$10^{-4} \text{M}$</td>
<td>↑ (28%)</td>
<td>60 min</td>
<td>Yes</td>
<td>Staskowski <em>et al.</em> (74)</td>
</tr>
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<td>β antagonists</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>propranolol hydrochloricium</td>
<td>0.01 mg ml$^{-1}$</td>
<td>↓ (53%)</td>
<td>3 h</td>
<td></td>
<td>Robson <em>et al.</em> (73)</td>
</tr>
<tr>
<td></td>
<td>0.1 mg ml$^{-1}$</td>
<td>↓ (67%)</td>
<td>3 h</td>
<td></td>
<td>Robson <em>et al.</em> (73)</td>
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<tr>
<td>H$_1$ antagonists</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>levocabastine</td>
<td>0.01%</td>
<td>↓ (13%)</td>
<td>20 min</td>
<td></td>
<td>Merkus <em>et al.</em> (63)</td>
</tr>
<tr>
<td>Drugs</td>
<td>Concentration</td>
<td>CBF (%) change</td>
<td>Time</td>
<td>Reversibility</td>
<td>Reference</td>
</tr>
<tr>
<td>-------------------------------------------</td>
<td>---------------</td>
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<tr>
<td>Ions/ionophores/inhibitors</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>potassium</td>
<td>2.5 mmol l⁻¹</td>
<td>↓ (15%)</td>
<td>3 h</td>
<td></td>
<td>Robson et al. (73)</td>
</tr>
<tr>
<td></td>
<td>7.5 mmol l⁻¹</td>
<td></td>
<td>3 h</td>
<td></td>
<td>Robson et al. (73)</td>
</tr>
<tr>
<td>4-Br-A23187</td>
<td>10⁻⁵ M</td>
<td>↑ (18%)</td>
<td>40 min</td>
<td></td>
<td>Di Benedetto et al. (87)</td>
</tr>
<tr>
<td>trifluoperazine</td>
<td>10⁻⁴ M</td>
<td>↓ (10%)</td>
<td>60 min</td>
<td>No</td>
<td>Di Benedetto et al. (87)</td>
</tr>
<tr>
<td>Arachidonic acid metabolites</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>prostaglandin F₂</td>
<td>10⁻⁸ M</td>
<td>↑ (12%)</td>
<td>20 min</td>
<td></td>
<td>Bonin et al. (20)</td>
</tr>
<tr>
<td>prostacyclin analog iloprost</td>
<td>10⁻⁸ M</td>
<td>↑ (13%)</td>
<td>20 min</td>
<td></td>
<td>Bonin et al. (20)</td>
</tr>
<tr>
<td>thromboxanc A₂ analog U46619</td>
<td>10⁻¹⁰-10⁻⁶ M</td>
<td>NE</td>
<td>20 min</td>
<td></td>
<td>Bonin et al. (20)</td>
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<tr>
<td>Hormones</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>insulin</td>
<td>1% (w/v)</td>
<td></td>
<td>30 min</td>
<td></td>
<td>Hermens et al. (77)</td>
</tr>
<tr>
<td>Neuropeptides</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>substance P</td>
<td>10⁻⁷ M</td>
<td>↑ (5%)</td>
<td>60 min</td>
<td>Yes</td>
<td>Staskowski et al. (74)</td>
</tr>
<tr>
<td></td>
<td>10⁻⁴ M</td>
<td>↑ (12%)</td>
<td>60 min</td>
<td>Yes</td>
<td>Staskowski et al. (74)</td>
</tr>
<tr>
<td>Mucolytics</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N-acetylcysteine</td>
<td>2 µg ml⁻¹</td>
<td>↓ (18%)</td>
<td>60 min</td>
<td>Yes</td>
<td>Stafanger et al. (25)</td>
</tr>
<tr>
<td></td>
<td>20 µg ml⁻¹</td>
<td>↓ (30%)</td>
<td>60 min</td>
<td>Yes</td>
<td>Stafanger et al. (25)</td>
</tr>
<tr>
<td></td>
<td>200 µg ml⁻¹</td>
<td>↓ (100%)</td>
<td>15 s</td>
<td>Yes</td>
<td>Stafanger et al. (25)</td>
</tr>
<tr>
<td>Absorption enhancers</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sodium taurodihydrofusidate</td>
<td>&gt;0.3% (w/v)</td>
<td>↓ (100%)</td>
<td>10 min</td>
<td>No</td>
<td>Hermens et al. (77)</td>
</tr>
<tr>
<td></td>
<td>0.3% (w/v)</td>
<td>↓ (36%)</td>
<td>30 min</td>
<td>Yes</td>
<td>Hermens et al. (77)</td>
</tr>
<tr>
<td>deoxycholate</td>
<td>0.3% (w/v)</td>
<td>↓ (98%)</td>
<td>10 min</td>
<td>No</td>
<td>Hermens et al. (77)</td>
</tr>
<tr>
<td>lauareth-9</td>
<td>0.3% (w/v)</td>
<td>↓ (100%)</td>
<td>10 min</td>
<td>No</td>
<td>Hermens et al. (77)</td>
</tr>
<tr>
<td>taurocholate, glycocholate</td>
<td>0.3% (w/v)</td>
<td>↓ (10-12%)</td>
<td>30 min</td>
<td>Yes</td>
<td>Hermens et al. (77)</td>
</tr>
<tr>
<td>Preservatives</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>chlorbutol</td>
<td>0.5%</td>
<td>↓ (100%)</td>
<td>5 min</td>
<td>Yes</td>
<td>Van de Donk et al. (70)</td>
</tr>
<tr>
<td>benzalkonium chloride+EDTA</td>
<td>0.006%+0.1%</td>
<td>↓ (35%)</td>
<td>20 min</td>
<td>No</td>
<td>Van de Donk et al. (70)</td>
</tr>
<tr>
<td>propylene glycol</td>
<td>20 µg ml⁻¹</td>
<td>↓ (30%)</td>
<td>20 min</td>
<td>Yes</td>
<td>Stafanger (78)</td>
</tr>
<tr>
<td></td>
<td>200 µg ml⁻¹</td>
<td>↓ (100%)</td>
<td>20 min</td>
<td>Yes</td>
<td>Stafanger (78)</td>
</tr>
<tr>
<td>thiomersal</td>
<td>25 µg ml⁻¹</td>
<td>↓ (100%)</td>
<td>5 min</td>
<td>No</td>
<td>Stanley et al. (58)</td>
</tr>
<tr>
<td></td>
<td>5 µg ml⁻¹</td>
<td>↓ (100%)</td>
<td>3 h</td>
<td>No</td>
<td>Stanley et al. (58)</td>
</tr>
</tbody>
</table>

↑increase, ↓decrease, NE no effect.
Beclomethasone dipropionate aerosol administered for 1 week did not alter saccharin transit time, whereas flunisolide nasal pump spray delayed transit, but this was attributed to the vehicle for flunisolide (60). In contrast, longer term studies by Scadding and colleagues (61) have demonstrated that 1 year treatment of perennial rhinitics with fluticasone propionate aqueous nasal spray led to a significant increase in nasal mucociliary clearance as assessed by saccharin test. This increase in clearance rate may have been the consequence of a beneficial action on the underlying rhinitis.

Although animal studies suggest an excitatory effect of histamine on ciliary activity (62), intranasal application of a suspension of the H1 anti-histamine levocabastine was found to have no effect on NMCC, in healthy humans assessed using the saccharin-dye test (63). Using the same method of assessment, chlorpheniramine administered orally was found to bring about more rapid NMCC as well as a reduction in expelled nasal secretions in healthy adults during rhinovirus infection (64). It was suggested that increased secretions as occurred during rhinovirus infection might impede normal mucociliary clearance and the effect of chlorpheniramine in reducing secretions might help preserve mucociliary clearance.

Topically administered a-adrenergic drugs are frequently used as nasal decongestants. In healthy human volunteers phenylephrine had no effect on nasal mucociliary transit times measured by the saccharin test, but increased CBF in samples of nasal cilia obtained 10 min after the topical application of 1% phenylephrine (65). The increase in CBF was attributed to the a-adrenergic stimulant effect of phenylephrine, while the lack of change in mucociliary transit time was thought to be due to the concurrent reduction in nasal mucus volume and increased mucus viscosity caused by the a-adrenergic stimulus. These findings are in agreement with a study in healthy humans in which topical treatment for 1 week with the a-agonist nasal decongestant phenylephrine, did not alter nasal mucociliary clearance assessed by the saccharin dye test (66). In neither of these studies was reference made to possible effects of preservatives in the nasal preparation. Van de Donk and colleagues (67) demonstrated prolongation of nasal saccharin clearance in man following the topical application of chlorbutol but further studies are required to exclude possible in vivo effects of other additives used in nasal preparations.

The effect of drugs on ciliary beat frequency of human nasal epithelial cells, in vitro

A number of studies have examined the effect of nasal preparations, on ciliary activity in vitro and have focused on the effects on CBF since, in addition to the rheological properties of mucus, this is thought to be an important factor influencing mucociliary clearance (68). Over the past two decades numerous studies have investigated the effect of these drugs on CBF of airway epithelial cells from both animal (69-71) and human (55, 72) tissue and several have suggested that CBF is neither different in humans compared with animals (27,70), nor in human nasal compared with bronchial epithelial cells (28). Indeed, some of these studies have suggested that the effects of drugs observed in different in vitro systems are comparable.

The effects, on CBF, of drugs not exhibiting anti-allergic properties are summarized in Table 1. Rutland and colleagues have investigated the effect of the commonly used local anaesthetic lignocaine, on the ciliary activity of nasal cells obtained by nasal brushing (45). They found that this agent does not inhibit CBF, in vitro, at doses which produce local nasal mucosal anaesthesia in vivo. Higher concentrations of lignocaine, however, led to high dose inhibition of ciliary activity.

Robson and colleagues (73) and Staskowski and colleagues (74) have studied the effect of the β2 agonists salbutamol and terbutaline, and demonstrated that these agents increased the CBF in nasal brushings and adenoid tissue, respectively, 1 h onwards. Studies by Robson and colleagues have further demonstrated that this effect could be blocked by use of the β2 antagonist propranolol. These findings are in accordance with those of Devalia and
co-workers, who studied the effects of salmeterol, salbutamol, terbutaline and propranolol (55,75), on CBF in bronchial epithelial cell cultures in vitro, and suggest that \( \beta_2 \) adrenergic agents may be of particular significance in enhancing mucociliary clearance, in vivo. These authors have also investigated the effect of methacholine on CBF in their model and demonstrated that this agent also enhances the CBF of bronchial epithelial cells, an effect that can be blocked by atropine (75). Studies by Van de Donk and colleagues (70), Robson and colleagues (73) and more recently Curtis and Carson (76) have shown that also sympathomimetics like naphazoline, xylometazoline and phenylephrine influence CBF of human nasal epithelial cells and may lead to a decrease in CBF at concentration as low as 0·1%. These findings suggest that adrenergic agonists may act directly on ciliated cells, and inhibit the frequency of ciliary beat.

Arachidonic acid metabolites have been shown to play an important role in the pathogenesis of certain airway diseases and consequently have been investigated for their effects on ciliary motility. Using epithelial cells from nasal brushings Bonin and colleagues (20) have recently reported that prostaglandin E\(_2\) (PGE\(_2\)) and the prostacyclin analogue Iloprost, both exert cilio-stimulatory effects at concentrations between 10\(^{-10}\)-10\(^{-6}\) M. Whilst the effect of Iloprost can be successfully blocked by the cyclooxygenase inhibitor indomethacin (10\(^{-6}\) M) the effect of PGE\(_2\) cannot. The authors conclude that arachidonic acid metabolites are important in the regulation of nasal ciliary activity.

The mucolytic agent N-acetylcysteine at concentrations which might occur locally in the nose, was found to reduce the CBF slightly in nasal cells obtained by curette technique (25). This effect was, however, fully reversible within 15 min. Similarly, antimicrobial and antiviral agents such as neomycin and ribavirin have also been shown to decrease the ciliary activity of nasal epithelial cells, in vitro, but this effect becomes manifest only after 5 h (22,23,58).

Since most commercially available nasal drops contain absorption enhancers and preservatives in addition to the drug itself, the effect of these additives has also been investigated on CBF in human nasal preparations in vitro. Hermens and colleagues (77) have performed extensive studies of the effect of these additives in human adenoid tissue and demonstrated that a large number of the preservatives and absorption enhancers decrease the ciliary beat frequency in this tissue. Taurocholate and glycolate at a concentration of 0·3% was shown to have a very mild effect on CBF as compared to the same concentration of deoxycholate, laureth-9 and sodium taurodihydrofusidate which caused marked reduction or cessation of CBF after 10 min of administration. Preservatives such as chlorbutol (70), propylene glycol (78) and thiomersal (58) also showed non-reversible detrimental effects on CBF after 5–20 min.

In recent years there has been a progressive increase in use of nasally administratable anti-inflammatory drugs with a concomitant increase in the number of studies investigating the effect of these preparations in vitro. The effect of anti-allergic drugs on the ciliary beat frequency of human nasal epithelial cells in vitro is shown in Table 2. Devalia and colleagues have investigated the effect of 10\(^{-5}\) M nedocromil sodium on the CBF of human nasal epithelial cells in culture and found no change in ciliary activity over a period of 24 h studied (57). In contrast, Stanley and colleagues (58) found that both betamethasone and betamethasone with neomycin drops were ciliotoxic to ciliated nasal epithelium. These authors concluded that this effect was likely to be caused by the preservative benzalkonium chloride and thiomersal. Further studies by the same group demonstrated that these preparations do not affect nasal clearance or ciliary beat frequency when administered topically, in vivo. Staafanger (78) reported similar findings when investigating the effects of beclomethasone dipropionate (BDP). This author demonstrated a dose-related irreversible decrease in CBF after perfusion of nasal epithelium cells with either BDP or flunisolide, thereby suggesting that caution should be taken when the dose and/or length of the treatment is considered in vivo, although topical corticosteroid treatment has not generally been accompanied by impairment of NMCC in vivo (58–61).

The importance of ciliary beat frequency and mucociliary clearance in disease states

Most studies assessing nasal mucociliary clearance in disease states have employed the saccharin test. Using this method, significant prolongation of clearance times has been noted in non-allergic rhinitis, nasal polyposis (79), asthma (80), bronchiectasis (81), ciliary dyskinesia (10), and in school children with positive skin test responses and bronchial hyper-responsiveness (11) as well as following infection with cold viruses (82). When clearance is abnormal, it is often not clear whether it is impairment of ciliary beating or abnormality of mucus which is responsible. In a study using the saccharin test in patients with perennial rhinitis or chronic infected rhinosinusitis with or without concomitant asthma or...
Table 2  The effect of anti-allergic drugs on the ciliary beat frequency of human nasal epithelial cells, *in vitro*  

<table>
<thead>
<tr>
<th>Drugs</th>
<th>Concentration</th>
<th>CBF (% change)</th>
<th>Time</th>
<th>Reversibility</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Betamethasone nose drop</td>
<td>1:2</td>
<td>↓ (100%)</td>
<td>5 min</td>
<td>No</td>
<td>Stanley et al. (38)</td>
</tr>
<tr>
<td>(betamethasone sodium phosphate+</td>
<td>1:10</td>
<td>↓ (100%)</td>
<td>90 min</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>benzalkonium+EDTA)</td>
<td>1:25</td>
<td>↓ (68%)</td>
<td>6 h</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:50</td>
<td>NE</td>
<td>6 h</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Betamethasone with neomycin nose drop</td>
<td>1:2</td>
<td>↓ (100%)</td>
<td>5 min</td>
<td>No</td>
<td>Stanley et al. (58)</td>
</tr>
<tr>
<td>(betamethasone sodium phosphate+</td>
<td>1:10</td>
<td>↓ (100%)</td>
<td>3 h</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>neomycin+thiomersal)</td>
<td>1:25</td>
<td>↓ (100%)</td>
<td>6 h</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1:50</td>
<td>NE</td>
<td>6 h</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Beclomethasone dipropionate</td>
<td>0.5 mg ml⁻¹</td>
<td>↓ (100%)</td>
<td>15 min</td>
<td>No</td>
<td>Stafanger (78)</td>
</tr>
<tr>
<td>(beclomethasone dipropionate+ benzalkonium)</td>
<td>0.1 mg ml⁻¹</td>
<td>↓ (100%)</td>
<td>60 min</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.05 mg ml⁻¹</td>
<td>↓ (50%)</td>
<td>60 min</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.005 mg ml⁻¹</td>
<td>↓ (25%)</td>
<td>60 min</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.0005 mg ml⁻¹</td>
<td>NE</td>
<td></td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Flunisolide</td>
<td>0.25 mg ml⁻¹</td>
<td>↓ (100%)</td>
<td>10 min</td>
<td>No</td>
<td>Stafanger (78)</td>
</tr>
<tr>
<td>(flunisolide+propylene glycol)</td>
<td>0.05 mg ml⁻¹</td>
<td>↓ (15%)</td>
<td>20 min</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.025 mg ml⁻¹</td>
<td>↓ (20%)</td>
<td>60 min</td>
<td>No</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.00025 mg ml⁻¹</td>
<td>NE</td>
<td></td>
<td>No</td>
<td></td>
</tr>
<tr>
<td>Nedocromil sodium</td>
<td>10⁻⁵ M</td>
<td>NE</td>
<td>up to 24 h</td>
<td>No</td>
<td>Devalia et al. (57)</td>
</tr>
</tbody>
</table>

↑increase, ↓decrease, NE no effect.
bronchiectasis, all patient groups had significantly longer clearance times than a group of healthy subjects (83). *In vitro* examination of cilia demonstrated that the abnormal clearance was not due to an intrinsic ciliary defect but probably to the result of a combination of mucus and ciliary factors *in vivo*. In a recent study, Veale and colleagues have investigated the variability in CBF in nasal tissue obtained by brushing in normal subjects and in patients with bronchiectasis (84). These authors demonstrated that although the CBF of the fastest beating cilia was similar in both groups, the CBF of the slowest beating cilia was lower and showed greater within subject variation in the bronchiectatic patients (84). Prolongation of nasal saccharin clearance has also been demonstrated in cystic fibrosis and is accompanied by normal ciliary beat frequency in samples of nasal epithelium, suggesting the presence of an abnormality of mucus *in vivo* (85).

**Conclusion**

With increased use of nasally administered pharmaceutical preparations it has become necessary to test these agents for both effectiveness and any associated toxic or adverse reactions. Although there are many reports of the effects of different therapeutic agents on human nasal mucociliary clearance, *in vivo*, and ciliary beat frequency, *in vitro*, it has not been possible to elucidate the precise mechanism/s underlying these effects. This is partly because of the lack of a direct correlation between the findings of the *in vivo* and *in vitro* studies. It is likely that this discrepancy is a consequence of the differences between the study systems employed. It is clear that factors such as temperature, humidity, pH, viscosity and ionic strength of the incubation medium, are critical for growth, maintenance and function of the cells *in vitro*, and consequently have to be controlled rigorously in all studies employing *in vitro* models. In contrast, the concentration and therefore the bioavailability of a therapeutic agent, *in vivo*, is likely to be influenced by the pharmacokinetic profile of the drug in the preparation, and as such will be affected by factors such as (i) dilution of the drug in nasal mucus, (ii) continual removal of the drug by mucociliary clearance, (iii) absorption and clearance of the drug into the bloodstream, and (iv) binding of free drug to proteins and other components of the nasal mucosa. On the basis of the evidence available, it is likely that the preservative agent/s in the nasally administered drug preparation will also be influenced similarly. However, due to the marked attenuating effect of the preservative agents observed on ciliary activity *in vitro*, there is a clear need for the effect of these agents to be investigated individually *in vivo*, before any firm conclusions can be drawn on the specific effect of any nasally administered therapeutic preparation on the mucociliary component.

**References**


