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Nasal mucociliary function: comparison of saccharin clearance with ciliary beat frequency

B.G. Moriarty, A.M. Robson, L.A. Smallman and A.B. Drake-Lee

Dept. of O.R.L., Queen Elizabeth Hospital, Birmingham, United Kingdom

SUMMARY

Mucociliary clearance as measured by saccharin clearance time is in part dependent on the physiological characteristics of mucus and is partly dependent on ciliary function. This study compared the relationship between saccharin clearance time and ciliary beat frequency in 44 patients attending a rhinology clinic. Whilst saccharin clearance time may be used as a screening test for ciliary function there was no clear linear relationship between mucociliary clearance and ciliary beat frequency.

INTRODUCTION

Mucociliary transport is the most important defence mechanism in the respiratory tract. This is dependent on an adequate quantity of mucus of appropriate viscoelasticity and on adequately functioning cilia. Micro-organisms are trapped by this moving carpet of mucus and are propelled from the upper and lower respiratory airways towards the pharynx where they are swallowed or expectorated (Sleigh et al., 1988). A defect in any part of this system, such as primary and secondary ciliary disorders and cystic fibrosis, may lead to recurrent or chronic respiratory disease. Nasal mucociliary clearance has traditionally been assessed by a number of methods. The most popular technique is the saccharin clearance test introduced by Andersen et al. (1974) and modified by Rutland and Cole (1981). This test is easy to perform, inexpensive and without complications.

Ciliary function alone can be measured in vitro by a photometric technique (Greenstone et al., 1984). Many workers have used saccharin clearance time as an index of mucociliary function in vitro (Rutland and Cole, 1981; Majima et al., 1986). To our knowledge the exact relationship between these two variables has not been firmly established.

The aim of this study was to determine saccharin clearance time (SCT) and ciliary beat frequency (CBF) in patients attending a rhinology clinic and to assess any correlation between the two indices.

MATERIALS AND METHODS

Patients

In 1987 a Rhinology Clinic was established at the Queen Elizabeth Hospital. Adult patients with chronic nasal symptoms are referred by their general practitioners and other ENT surgeons in the West Midlands Region. A questionnaire on the patient's symptoms and past medical history is completed by the surgeon followed by a thorough clinical examination. The subsequent investigations include: relevant blood test, X-rays, skin allergy tests, saccharin clearance test and measurement of CBF. Patients were included in the study if there was no gross septal deformity, no mucopus in the nasal cavity, no obstructing nasal polyps and the sinus radiographs were normal or showed minimal thickening as previously defined (Pfleiderer et al., 1984).

Normal Controls

Six medical students were used as controls. They had no history, or evidence of nasal disease and negative skin tests.

Saccharin clearance test

One-quarter of a Hermesetas saccharin tablet was placed under direct vision using Tilley nasal dressing forceps on the medial aspect of the inferior turbinate 1 cm behind the anterior margin. Each subject was asked not to sniff, blow their nose, eat, drink and to hold their head level for the test. The time taken to notice the first sensation of sweetness at the back of the mouth was recorded to the nearest minute. In all instances these patients had no nasal obstruction. The test was performed in an identical manner by the same observer (AD-L) on the left side of the nose in all patients. Five patients had saccharin clearances prior to this study and transit times were repeated twice. All clearances were within a minute confirming the reliability of their method as has been documented elsewhere (Stanley et al., 1984). Confirmation of the reliability comes also from the close clustering of the normal values in the controls.

Nasal brushing and ciliary beat frequency

A 1.73 mm diameter cytology brush (Medi-Tech) was inserted approximately 5 cm into the right nasal cavity after initial wetting in tissue culture medium (Medium 199 with Hepes and Earles salts, Flow laboratories). The brush was moved rapidly antero-posteriorly along the lateral wall of the cavity and the sample dislodged into 2–3 cc of tissue culture medium by brisk agitation. This procedure was repeated until sufficient fragments of epithelium were collected. These were transferred from the tissue culture medium with a Pasteur pipette to a welled microscope slide (26 x 45 mm, Chance Propper Limited) and gently sealed

with a coverslip. The CBF was measured using a photometric method, modified after Greenstone et al. (1984). The slide was placed on an electrically controlled warm stage at 37 °C mounted on a Zeiss Universal microscope. A Zeiss SF photometer attached to the head of the microscope transduced changes in light intensity into an electrical signal. Beating cilia were easily seen at a magnification of x400 by bright field illumination, positioned so they intermittently obstructed the passage of light through a small diaphragm (3.14 μm) into the SF photometer. The transduced electrical signal was amplified and displayed on an oscilloscope screen (Tektronix 564B). Black and white photographs of the traces were then taken using a Minolta SPT 101 camera to provide permanent record of traces which were analyzed later. The beat frequency of the most vigorous cilia attached to epithelia was measured in more than 10 areas and the mean was calculated. All the beat frequencies were measured within two hours of the sample being taken.

Statistical analysis

On the basis of the results, the 43 patients were divided into the following groups: saccharin transit times less than or equal to 12 minutes and those above this value: CBF less then or equal to 11 b/s and those above this reading. These groups were compared by a Chi squared test with Yates correction for small numbers.

RESULTS

44 patients attending this clinic were suitable for the study, these included 20 males and 24 females, age range from 19 to 76 years. Four patients had non-obstructing nasal polyps, the remaining cases were equally divided between cases with allergic and non-allergic rhinitis. None had chronic infective rhinosinusitis.

One patient did not taste the saccharin after 30 mins and did not attend for repeat testing, however the CBF in this patient was 16 b/s.

The mean SCT in 43 patients was 12 mins with a range of 6 to 25 mins. It varied between 7 and 11 mins with a mean of 8 mins in controls.

The mean CBF in patients was 11 b/s with a range of 5 to 19 b/s. The CBF in controls was 14 b/s with a range of 13 to 16 b/s.

Table 1 shows the statistical correlations of both SCT and CBF in controls and patients. SCT was significantly shorter in healthy controls and the CBF was significantly faster (both $p < 0.001$).

The results in patients were plotted on a scatter diagram (Figure 1). This showed no clear linear relationship between the two variables which was confirmed by the Chi squared testing. The correlation coefficient was $r = -0.33$, and a two tailed test of significance, $0.05 > p > 0.01$. There was no correlation between high and low transit times and high and low CBF (correct Chi squared testing).

Table 1. Statistical correlation of both SCT and CBF in controls and patients.

	SCT (mins)		CBF (b/s)	
	controls	patients	controls	patients
mean	8	12	14	11
SD	2	5	1	3
SEM	1.5	1	0.5	0.5
	$p < 0.001$		$p < 0.001$	

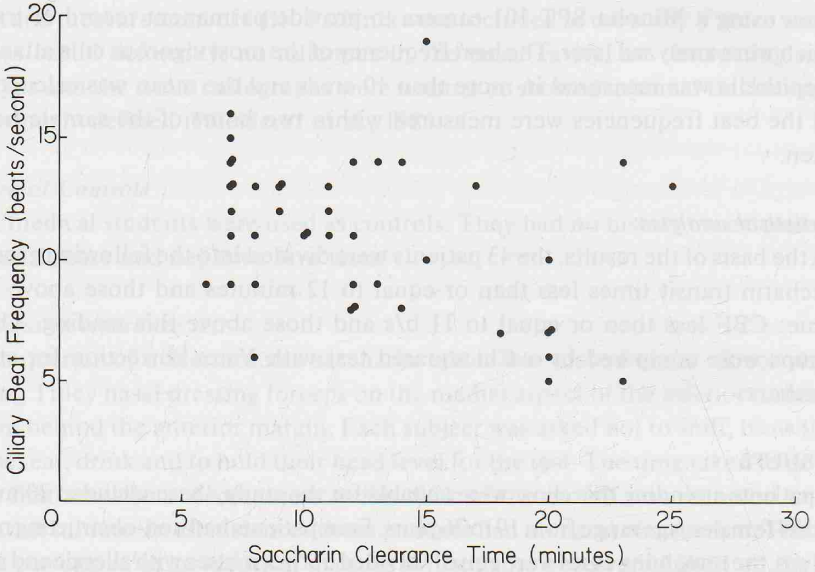

Figure 1. Scatter diagram to show relationship between saccharin clearance time and ciliary beat frequency.

DISCUSSION

Normal mucociliary transport depends on structurally and functionally intact ciliated epithelium as well as the quantity and rheology of the mucus. Cilia beat in a bi-layered fluid system with a watery periciliary fluid lying below a high viscosity mucus layer (Sleigh et al., 1988).

Mucociliary clearance is a function of CBF and the quantity and viscoelastic properties of mucus. Saccharin clearance and CBF measure different parameters. CBF measured in vitro only looks at one aspect of mucociliary transport. Many observers have relied on measurement of saccharin clearance as a direct index of CBF although the exact relationship has never been established.

A number of techniques have been used to assess nasal mucociliary clearance.

These include visible markers (dye solutions, coloured powders, pieces of paper, aluminium discs) and radio-labelled markers (Andersen and Proctor, 1983). The method which has gained the most popularity is the saccharin clearance test introduced by Andersen (1974) and modified by Rutland and Cole (1981). Ciliated epithelium can be obtained directly by a non-invasive brush technique and measurement of the CBF in vitro is possible using a photometric method. Healthy subjects with normal mucociliary clearance taste the saccharin within 20 mins (Stanley et al., 1985). The variations in the values of SCT may be due to technical inconsistencies. These may be minimized by either repeating the test which requires a number of visits or by evolving a technique to give as little error as possible. We have modified the test by using a single fragment which allows accurate placement of a readily absorbable particle at the same place in all patients. Brushing powder places a variable quantity of volatile powder over a large area which may initiate sneezing. Patients who fail to taste saccharin after at least 30 mins should be assessed to see if they can taste saccharin when it is placed directly on the tongue. The test should be repeated and if the clearance time is still prolonged a nasal brushing taken to look for ciliary abnormalities. A close correlation between SCT on both sides of the nose has been established in patients without nasal obstruction (Stanley et al., 1984). Normal cilia beat at frequency of 11–17 b/s. In our series of 44 consecutive patients the results for mean SCT (12 mins) and mean CBF (11 b/s) are similar to findings by other workers (Rutland and Cole, 1981; Katz et al., 1987). The patient group was heterogeneous, but it is possible to state that SCT was longer in patients than in controls and that CBF was slower, which suggests that mucociliary clearance may be affected adversely in non-suppurative disease.

Our results plotted on a scatter diagram (Figure 1) show no close linear correlation between SCT and CBF. There are a number of possible explanations for this finding. In the first instance the mucociliary transport depends not only on the frequency of the beating cilia but also on quantity and quality of the mucus. These properties of mucus may vary from individual to individual and this will upset the relationship between the SCT and CBF. However, if the cilia are immotile of dyskinetic the overall product of CBF and mucus properties will still be low. This accounts for the prolonged SCT in these individuals (Greenstone et al., 1988). Another possible explanation for the discrepancy between SCT and CBF may be that the nasal brushing is taken from the upper part of the nose where cilia are abundant, whereas the saccharin dissolves and is transported in the periciliary fluid and mucus in the inferior part of the nose. The in vitro CBF may not necessarily be the in situ CBF since other factors may affect in vivo CBF. Visual display on an oscilloscope gives a limited sample which makes it necessary to have 10 readings to obtain the CBF. Since this study we have developed a computer programme which controls an analogue to digital converted and analyses the signal by

Fast Fourier transformation. It is now possible for us to give accurate information on the intra- and inter-individual variations (unpublished data).

In conclusion, although direct comparison of the results is not possible, some conclusions can be made about the two techniques. SCT is a very cheap, non-invasive and easily repeatable technique that is a useful screening test and can be performed in every clinic by those who are able to undertake rhinoscopy. Few departments have the apparatus required to study CBF which measure one aspect of ciliary function alone. No information is given on the co-ordination or efficacy of the beat, but if both tests are normal then they probably exclude a mucociliary problem. These tests are therefore complimentary and measure different aspects of mucociliary function which is confirmed by the correlation number which as expected was not linear.

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Dr. L.A. Smallman
Dept. of Pathology
The Medical School
University of Birmingham
B15 2TJ United Kingdom