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Effect of surface-active substance on nasal mucociliary clearance time: A comparison of saccharin clearance time before and after the use of surface-active substance*

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SUMMARY *Mucociliary clearance measured by saccharin clearance time is depending on ciliary function and on the physiological characteristics of mucus. The aim of this study was to determine whether the application of surface-active substances changed the mucociliary transport time. Twenty healthy persons were manually sprayed with surface-active substance in one of their nose cavities. The saccharin clearance time was measured before spraying and statistically compared with saccharin clearance time after spraying. Saccharin clearance time was significantly shortened immediately after spraying with surface-active substances. This difference was not found 2 h later. Our assay indicates that surfactant increases the rate of mucociliary transport in the upper respiratory tract.*

Key words: mucociliary clearance, surface-active substances, surfactant, nasal mucosa

INTRODUCTION

Mucociliary transport is the most important defence mechanism in the respiratory tract. Mucus is secreted onto the mucosal surface of the respiratory tract. The airway secretions, also known as respiratory mucus forms a biphasic fluid composed of an aqueous sol phase ("epithelial lining fluid" or "periciliary fluid") in which the cilia beat and relax, and a gel phase located on the tips of the cilia. The latter is propelled by the tips of the cilia and mechanically eliminated by means of mucociliary clearance (Phipps, 1981; Sleigh et al., 1988; Puchelle, 1992).

Airborne particles that are trapped on the mucus are propelled from the upper and lower respiratory airways to the pharynx, where they are swallowed or expectorated. This mucociliary transport is depending partly on the presence of sufficient quantities of mucus with the appropriate visco-elasicity, and partly on adequately functioning of cilia (Phipps, 1981; Sleigh et al., 1988). Mucus is essential for particle transport, and cilia – even although they will continue beating – are unable to transport particles in the absence of mucus (Silberberg et al., 1977). The requirement of the mucus is physical rather than chemical; since chemically unrelated substances can substitute for mucus as long as they have a minimum degree of cross-linking and have physical properties that are within certain limits (King, 1974). Mucus has the properties of both a liquid (viscosity) and of a solid (elasticity). The frog palate has been used for studying mucus transport rate and it has been found that mucociliary transport rate was maximum at certain low values of both viscosity and elasticity. It has been demonstrated that there is a sharp increase in mucociliary transport rate with increase in viscosity and elasticity up to those certain low values for both viscocity and elasticity, and at the higher values of both viscocity and elasticity there is a slow decrease in mucocilliary transport rate with decreasing viscosity and elasticity (Dulfano and Adler, 1975; Majima et al., 1986).

Other possible properties which could influence mucociliary transport time are surface-active substances present in the respiratory tract secretions. The aim of this investigation was to determine whether addition of surface-active substance influences mucociliary transport.

MATERIAL AND METHODS

Twenty healthy volunteers, 14 women and 6 men, with a mean age of 39 years (26-52 years) were tested. All were

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non-smokers without upper airway infections for at least one month and without known allergy. Clinical upper airway inspection was normal, especially without mechanical obstructions such as gross septum deviations or polyps.
The subjects were informed about the investigation and the investigation was accepted by the Local Ethical Committee.
Mucociliary clearance time was measured by saccharin clearance time. One-quarter of a saccharin tablet (Hermesetas[R]) was placed under direct vision on the medial-inferior aspect of the inferior turbinate, 1 cm behind the anterior end. The volunteers were told not to sniff or blow their noses. The time elapsed before the volunteers experienced the sweet taste was noted. Two hours later another test was done.
Then, on the same time of day, 1–3 days later the same nose cavity was sprayed with a manual spray containing surface-active substance. Two puffs were given, each delivering 0.05 ml. The aqueous mixture contained sodium chloride (9 g/l), 85% glycerol (3 g/l) and polysorbate-80 (2 g/l) and was delivered in bottles with manual spray by the pharmacy of Odense University Hospital.
The saccharin clearance time was determined immediately after spraying and 2 h after spraying.

RESULTS

The results are shown in Table 1. There were no differences when comparing the first and second saccharin clearance time. After spraying with surface-active substance 1–3 days later the saccharin clearance time was shortened significantly when measured immediately after spraying, but 2 h after spraying no differences were observed.

Table 1. Volunteers' number, sex, age and mucociliary clearance time (MCT) in min assayed twice with 2-h intervals before spraying with surface-active substances, and assayed twice with 2-h intervals after spraying with surface-active substances (Wilcoxon's signed rank test: $p<0.01$ for comparing A and C; $p>0.1$ for comparing B and 0; and $p>0.1$ for comparing A and B).

			MCT (min.) before spraying		MCT (min.) after spraying	
volunteer number	sex	age	1. assay (A)	2. assay (B)	1. assay (A)	2. assay (B)
1	F	52	11	10	10	12
2	F	36	9	14	10	16
3	M	36	10	14	12	17
4	F	45	14	12	9	6
5	F	44	11	13	12	14
6	M	43	15	17	14	18
7	M	38	16	15	22	12
8	F	36	13	18	9	14
9	F	26	19	15	12	8
10	M	38	15	10	8	9
11	F	39	12	11	10	11
12	M	45	34	24	32	28
13	F	42	11	14	6	7
14	F	43	13	14	10	10
15	F	33	18	22	15	16
16	F	42	28	24	23	30
17	F	43	12	7	8	12
18	F	33	10	7	6	11
19	M	45	10	15	11	15
20	F	32	12	9	8	12
Median values (and interquartiles)			13 (11-16)	14 (10-15)	10 (8-14)	12 (10-16)

DISCUSSION

The alveolar surfaces are lined with surfactant, a surface-active material mainly consisting of phospholipids that are synthesized by the Type II pneumocysts. It reduces surface tension and counteracts alveolar collapse (Bangham, 1987). The same phospholipids have also been described in secretions from the central airways, the nasal cavity, and the Eustachian tube (Birken and Brookler, 1972; Hills, 1984; Svane-Knudsen et al., 1988), but the composition of phospholipids seems to be organ-specific (Svane-Knudsen et al., 1988). The role of phospholipids in mucus from the central airways and in mucus from the nose is not clear. Theoretically, it may provide adequate physiological properties of mucus and hereby influence the mucociliary transport time. Gunnarsson et al. (1984) studied the *in vivo* influence of bromhexine (NA 872; bromhexine metabolite VIII) and the ß2-receptor agonist NAB 365 (clenbuterol) on mucociliary activity using rabbits. They concluded that the three substances had no effect on ciliary beating activity in rabbits *in vivo*, but underlined that this did not exclude an effect on mucociliary clearance. In their discussion they emphasize that the finding that NA 872 should favour mucociliary clearance could be ascribed to its ability to stimulate production of phosphatidylcholine (Kapanci, 1983), a potent surface-active substance. In the investigation by Gunnarsson et al. (1984) it was shown that the mucociliary beat frequency did not change after administration of NA 872, which could support the hypothesis that surfactant first of all reduces the adhesive forces and hereby facilitates mucocilary transport.
The influence of surfactant on mucociliary transport *in vitro* has been studied by Allegra et al. (1985). They used excised frog palate without capacity to secrete surfactant and mechanically depleted the palate from mucus. Then, the excised palate was sprayed with surfactant and its effect on mucociliary transport, restored by the application of mucus, was compared with that of sprayed saline. Whereas saline induced a constant decrease in restored transport rate, surfactant caused an increase in 5 out of 6 experiments. The difference between the two treatments were highly significant, with the highest mucociliary transport rate when surfactant was sprayed on the excised frog palate. Allegra et al. (1985) conclude that increased levels of surfactant may have an anti-adhesive effect on cilia-mucus coupling.
In our *in vivo* study we wanted to test whether addition of surface-active substance to the secretion of the nose changed the mucociliary clearance time as measured by saccharin clearance time. The surface-active substance we used was polysorbate-80, which is a non-ionic surfactant (Widdicombe and Davies, 1988). The mixture containing polysorbate-80 is the the same as described by Widdicombe

and Davies (1988) called Sonarex, except that we omitted benzalkoniumchlorid because of its possible inhibition of ciliary activity (Toremalm, 1985). In our study we used the nose as test organ. We used saccharin clearance time since it is widely accepted as a measure of mucociliary clearance.
The mixture containing surface-active substance was delivered in bottles with manual spray, because we assumed that spraying would give the best distribution of the surface-active substance, and that manual spraying would result in the least mechanical manipulation. By using manual spraying we avoided high-speed deposition and expellent gasses.
The results showed that the mixture containing surface-active substance temporarily reduced the mucociliary clearance time. After 2 h there were no differences, and on that account we assume that the surface-active substance we used must have been located in the gel phase of the mucus and cleared with it.
One of the role of phospholipids in mucus from the central airways and nose may be a reduction of the adhesion of mucus to cellular surfaces (Reifenrath, 1983; Gunnarsson, 1984; Allegra et al., 1985). Furthermore, phospholipids in mucus may have an anti-agglomerating effect upon the mucus particles (Schlimmer et al., 1983). The increased mucocilliary transport rate we found could be explained by reduced adhesion of mucus/cilia coupling.

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