

Short communication

The effect of antiallergic intranasal formulations on ciliary beat frequency of human nasal epithelium *in vitro*

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The nasal mucociliary clearance is an important defense mechanism of the upper respiratory tract. It is known to be influenced by many pharmacologic substances. We investigated the effects of three topical intranasal antiallergic formulations containing disodium cromoglycate (DNCG), dimethindene maleate (DMM), and azelastine-HCL (AZL) on nasal ciliary beat frequency (CBF) *in vitro*. Nasal ciliated cells were harvested from 16 healthy volunteers. Cells were diluted 1:10 in culture medium and incubated with a placebo formulation (PLAC), containing 0.022% benzalkonium chloride, which was part of all formulations, a registered 2% formulation of DNCG, a 0.1% formulation of DMM, and a registered 0.1% formulation of AZL. After an incubation period of 20 min, CBF was registered by a photoelectric measurement device. Under control conditions (CONTROL), CBF was 11.4 ± 1.3 Hz. PLAC reduced CBF to 9.7 ± 2.3 Hz (NS). DNCG reduced CBF to 9.7 ± 2 Hz (NS). DMM reduced CBF to 7.2 ± 1.7 Hz ($P \leq 0.05$ vs CONTROL, NS vs PLAC), and AZL reduced CBF to 0.9 ± 1.8 Hz ($P \leq 0.001$ vs CONTROL, $P \leq 0.001$ vs DNCG, $P \leq 0.001$ vs PLAC). In conclusion, a possible influence of antiallergic intranasal formulations on nasal ciliary function has to be considered in clinical application.

Key words: azelastine; ciliary beat frequency; dimethindene maleate; disodium cromoglycate; intranasal antiallergic therapy.

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The mucociliary clearance is an important defense mechanism of the nasal respiratory epithelium (1). This complex system, consisting of an accurately balanced mucous layer moved by intact, metachronously beating ciliated cells, has been found to be influenced not only by pathologic conditions, e.g., acute or chronic rhinitis (2, 3), or nasal allergies (4), but also by many pharmacologic substances (5, 6).

Over the last decades, several new pharmacologic substances have been introduced successfully for topical intranasal therapy of seasonal or perennial allergic rhinitis (7, 8). Due to their well-known effects on local allergic symptoms, modern nasal sprays containing the mast-cell stabilizer disodium cromoglycate, or the H_1 -antagonist azelastine (9–11) are among the most widely used pharmaceutical formulations. However, very little is known about their effect on nasal mucociliary function.

In the present study, we evaluated the effect of three different antiallergic intranasal formulations on ciliary beat frequency (CBF) as a key parameter of mucociliary function of nasal respiratory epithelium *in vitro*.

Material and methods

The study was carried out on human nasal respiratory epithelial cells *in vitro*. A summary of the experimental design is given in Fig. 1.

Nasal respiratory epithelial cells were harvested with a small ear curette from the posterior part of the inferior nasal turbinate of 16 healthy volunteers. No local anesthesia was used. A cell suspension was prepared by shaking the cell-bearing curette in 700 μ l of a sterile cell-culture medium (Dulbecco's modified Eagle's medium, Sigma Chemicals Co., St Louis, MO, USA). The cell

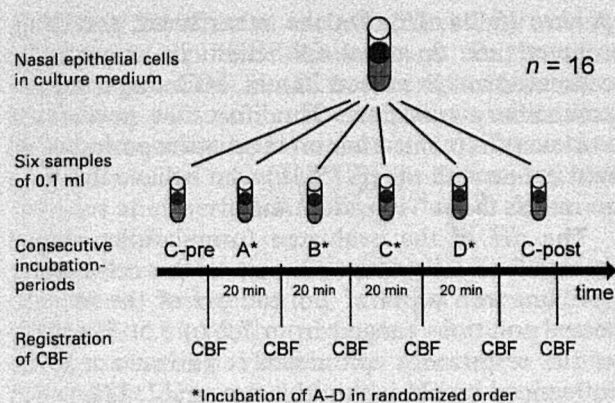


Fig. 1. Summary of experimental design. Consecutive incubation of two controls (C-pre and C-post) and four test solutions. Incubation of test solutions (A–D) in randomized order.

suspensions and all subsequent preparations were kept at a constant temperature of 37°C during the entire evaluation period.

After microscopic screening for cell content and cell vitality, each of the cell suspensions was divided into six samples of 100 µl each. Two samples served as controls and were evaluated at the beginning (C-pre) and the end (C-post) of the evaluation period. The other samples were consecutively incubated with 10 µl of one of the test solutions for 20 min. According to the experimental design, the four test solutions were transferred to neutral bottles and labeled from "A" to "D". The processing order of the different test solutions was randomized. Experiments were evaluated by an investigator blinded to the particular experimental condition. Therefore, the investigator was not aware of the content of the investigated sample.

Measurement of CBF was done at the end of each incubation period ($t=20$ min) by a photoelectric method described in detail elsewhere (1, 12). For this purpose, a small amount of each suspension was placed in a Fuchs-Rosenthal chamber and put under the microscope. In each suspension, 10 active cells were evaluated for 10 subsequent seconds.

The following antiallergic formulations were investigated: a registered 2% disodium cromoglycate

(DNCG) formulation; a new formulation, containing 0.1% of the H_1 -antagonist dimethindene maleate (DMM), which has not yet been introduced for topical intranasal therapy; and a registered 0.1% azelastine-HCl (AZL) formulation. For quantification of the effect of the preservative benzalkonium chloride, which was part of all evaluated formulations, a placebo formulation (PLAC) containing 0.022% benzalkonium chloride was evaluated as well. The pH values of the pure evaluated formulations and of their *in vitro* tested solutions were measured at 37°C by a digital pH meter 646 (Fa. Krick, Germany). The concentrations of the anti-allergic substances, the concentrations of benzalkonium chloride, and the pH of pure formulations and their solutions are given in Table 1.

The four treatments were applied on the same healthy volunteers' material by a randomized block design. For testing the stability of the cell suspensions, the CBF of two control cell suspensions in pure culture medium (C-pre and C-post) was measured before and after incubation of the four test solutions, respectively. Correlation between C-pre and C-post was evaluated by determination of the correlation coefficient and by comparison of the mean CBF values with the two-sided *t*-test. The mean CBF values of the four treatment groups were compared with each other and with a control value (CONTROL). To obtain this CONTROL, C-pre and C-post were averaged. For analysis of the four treatment groups, a parametric test was not applicable due to many zero measurements in one group. Thus, the nonparametric Friedmann analysis was used. The levels of significance are given with each result.

Results

Under control conditions, the mean CBF of C-pre was 11.2 ± 1.2 Hz (mean $\pm$ SD) and shifted to 11.6 ± 1.5 Hz for C-post ($P \leq 0.05$). The correlation coefficient between C-pre and C-post was $r=0.92$. The mean value of CONTROL was 11.4 ± 1.3 Hz.

The results of the mean CBF values of the four treatment groups are given in Fig. 2. The PLAC formulation reduced CBF to 9.7 ± 2.3 Hz (NS vs

Table 1. Concentrations of antiallergic substances, benzalkonium chloride, and pH of evaluated intranasal antiallergic formulations before and after dilution in DMEM (Dulbecco's modified Eagle's medium) with pH 7.6

Formulation	Antiallergic substance and concentration	Benzalkonium chloride concentration	pH of pure intranasal formulation	pH of <i>in vitro</i> tested 1:10 solution in DMEM
PLAC	None	0.022%	6.2	7.2
DMM	0.1% dimethindene maleate	0.022%	6.2	7.2
DNCG	2% disodium cromoglycate	0.01%	5.5	7.5
AZL	0.1% azelastine-HCl	0.0125%	6.8	7.5

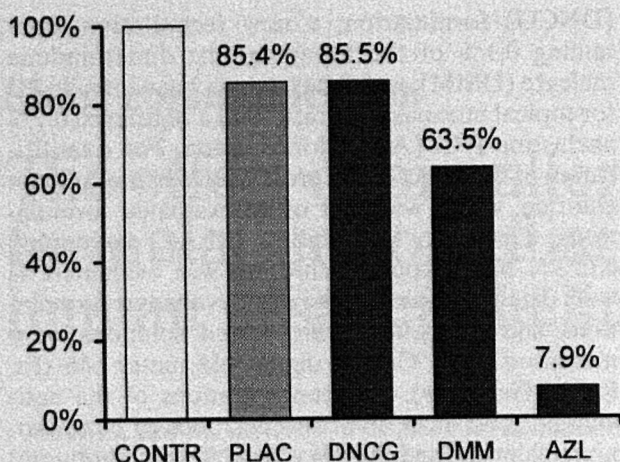


Fig. 2. Relative CBF of evaluated formulations and placebo formulation compared with CONTROL.

CONTROL). The DNCG formulation reduced CBF to 9.7 ± 2 Hz (NS vs CONTROL, NS vs PLAC). The DMM formulation reduced CBF to 7.2 ± 1.7 Hz ($P \leq 0.05$ vs CONTROL, PLAC, NS vs DNCG). The AZL formulation reduced CBF to 0.9 ± 1.8 Hz ($P \leq 0.001$ vs CONTROL, $P \leq 0.001$ vs PLAC, $P \leq 0.001$ vs DNCG, NS vs DMM); in 9 of 16 cases, we found complete cessation of ciliary movements.

Discussion

In patients with nasal allergies, CBF is known to be reduced, and nasal mucociliary transport time (NMTT) is known to be prolonged independently of an acute allergen challenge (4, 13). Ciliary activity was found to be more reduced with increasing duration of the morbid period (4). After acute allergen challenge, the NMTT of patients with nasal allergies is individually prolonged, essentially because of a sometimes severely increased nasal secretion (13). This individual variation of the mucociliary function in patients with nasal allergies has to be considered, making it unlikely for one to obtain statistically reliable results in a clinical study for comparative investigation of drug effects. Therefore, we decided to investigate CBF as a critical factor of mucociliary function *in vitro*.

The vitality and the stability of the ciliated cells over the entire experimental period were demonstrated by comparison of the C-pre and C-post measurements, showing a substantial correlation. The test solutions were diluted (one part test solution to 10 parts cell suspension) according to considerations by Deitmer & Scheffler earlier for adapting *in vivo* intranasal dilution of applied nasal sprays on the nasal mucosal surface to *in vitro* conditions (5). Drug-induced changes on CBF are often observed within 5–20 min after incubation

in vitro (5, 14, 15). On the other hand, the drug-contact time on nasal epithelium *in vivo* can be estimated not to exceed 20 min, assuming a regular mucociliary transport. Therefore, we performed our measurements after an incubation period of 20 min in line with others (5, 16) who believe this time to match the *in vivo* situation.

The pH of the evaluated formulations ranged from 5.5 to 6.8. After dilution in the cell-culture medium with a pH of 7.6, the pH of the *in vitro* tested solutions ranged from 7.2 to 7.5. The CBF of the respiratory epithelium is known not to be influenced by pH within this range (17, 18), which matches the pH of the nasal mucus *in vivo* (19).

The reduction of CBF caused by the common preservative benzalkonium chloride is well known (5, 20). Benzalkonium chloride was part of all evaluated formulations. Thus, correlation of the effect of the placebo formulation, containing 0.022% benzalkonium chloride, with the tested antiallergic formulations, which contained the same concentration (DMM) or a reduced concentration (AZL, DNCG) of benzalkonium chloride, was of special interest for quantification of the effects of the three evaluated active antiallergic substances on CBF.

The DNCG formulation induced no reduction of CBF compared to the placebo formulation. This is consistent with findings of other investigators (21). Thus, our results confirm that the mast-cell stabilizing agent DNCG seems to have no adverse effects on ciliary function *in vitro*.

DMM is a widely used H_1 -antagonist for oral administration or topical use on the skin. It has been reported to reduce nasal allergic symptoms significantly after systemic application (22, 23). However, nothing is known about its effect on the mucociliary system so far. In our investigations, the new intranasal DMM formulation induced mild reduction of CBF, which was statistically significant when compared to the control solution and not significant when compared to the placebo formulation. In conclusion, we were unable to find a substantial effect of DMM on ciliary activity of nasal epithelium *in vitro*. These findings may contribute to the clinical introduction of the new topical DMM formulation.

AZL is an antiallergic drug for topical (intranasal) or systemic (oral) use. It has been demonstrated to have H_1 -antagonizing effects as well as other antiallergic effects on antigen-induced inflammatory mediators and inflammatory cellular infiltrate (24, 25). In our study, we evaluated an azelastine formulation for intranasal application. However, it significantly reduced ciliary activity with complete cessation of all ciliary movements in several cases. In the literature, controversial results

regarding the effect of azelastine on ciliary activity have been reported. While Su & Po found a reduction of CBF in rat trachea specimens after incubation with similar concentrations of another intranasal azelastine formulation (14), Tamaoki et al. observed an increased CBF in human tracheal biopsies after perfusion with a 10^{-4} mol/l azelastine solution (15).

In contrast to the controversial findings for azelastine, levocabastine, another topical H_1 -antagonist currently promoted for intranasal therapy of allergic rhinitis, was reported to induce only slight depression of nasal ciliary activity *in vitro* and to have no substantial effect on NMTT *in vivo* (16).

In conclusion, the evaluated intranasal anti-allergic formulations have a substantially different influence on nasal CBF *in vitro*. The observed effects varied from no depression to severe depression of ciliary activity. A possible influence of antiallergic intranasal formulations such as azelastine on ciliary function has to be considered in clinical application, but further investigations of *in vitro* and *in vivo* effects of intranasal antiallergic formulations on mucociliary function are required to clarify this point. Because of the widespread use of these substances by patients with seasonal or perennial allergic rhinitis, these studies are of major importance for their safe, long-term application.

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