Influence of Preservatives and Topical Steroids on Ciliary Beat Frequency In Vitro

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**Objective:** To measure the influence of topical steroids and the preservative potassium sorbate on the ciliary beat frequency (CBF) of human nasal mucosa in vitro.

**Design:** In vitro study of cultured ciliated cells of human nasal mucosa.

**Methods:** Human nasal mucosa was removed endoscopically and cultured for 10 days. Cell cultures with ciliated cells grown on an object slide were exposed to benzalkonium chloride and topical steroids in an exposure chamber. The CBF was measured with a photometer.

**Results:** The preservative potassium sorbate did not influence CBF in different concentrations. The glucocorticoid budesonide spray containing potassium sorbate did not affect CBF at 10% dilution and showed moderate reversible decrease of CBF at 50% dilution. The glucocorticoid sprays fluticasone propionate and mometasone furoate containing the preservative benzalkonium chloride caused a reversible decrease of CBF at 10% dilution and a complete irreversible standstill at 50% dilution.

**Conclusions:** In vitro, the steroid sprays containing fluticasone or mometasone, both with benzalkonium chloride, caused slowing or standstill of CBF depending on the concentration. The isolated preservative potassium sorbate and the budesonide nasal spray containing this preservative did not have negative influence on CBF in vitro. Potassium sorbate can therefore be considered harmless to the motility of ciliated cells.


**METHODS**

During routine endonasal endoscopic sinus surgery for chronic sinusitis or nasal polyposis, mucosa was harvested from the uncinate process or middle turbinate if there was no sign of polyps, mucosal edema, or pathologic mucous. No patient had previous endonasal surgery. The samples were immediately placed in isotonic sodium chloride solution (normal saline) and transported to the laboratory. The tissue sample of each patient was sufficient for 8 to 10 cell cultures, which were kept in nutritive medium (M199; Sigma, St Louis, Mo) with penicillin and streptomycin and incubated in an atmosphere of 5% carbon dioxide, 95% air, and 100% humidity.

After 10 days, cells were spread on an object slide to a field size up to 4 × 8 mm. On average, 3 of 10 cell cultures contained ciliated cells. For visualization, we used a Zeiss Axiomat reversed microscope (Carl Zeiss, Oberko-chen, Germany). The object slide with the cultivated cells fixed on the surface was placed in a 30-mm-diameter waterproof chamber on the microscope (Figure 1). With a special minipump it was possible to rinse the cell culture with fluid and maintain a constant fluid level of 1.5 to 2 mm in the exposure chamber. With this system, the fluid surrounding the cell culture could be changed without drying, and therefore damage to the cilia was prevented. Thus it was possible to rinse the cell culture with either neutral Tyrode solution (137 mM sodium chloride, 2.7 mM potassium chloride, 10 mM HEPES, pH 7.4) or a solution containing 35% polyethylene glycol 400.

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1.8 mM calcium chloride, 2.2 mM sodium bicarbonate, 0.4 mM sodium hydrogen phosphate, 1.1 mM magnesium chloride, and 5.6 mM glucose at pH 7.2), which does not influence the CBF, or with a solution containing nasal spray or preservative alone.

The nasal sprays (Table 1) and the isolated preservatives were tested in 2 series of different dilutions with Tyrode solution (Table 2 and Table 3). The dilutions were designed to imitate the in vivo situation, where topical medications are dispersed on the surface of the nasal mucosa and are therefore diluted.10,11 The nasal sprays were tested in 10% dilution (1 part nasal spray to 9 parts Tyrode solution) and 50% dilution (1 part nasal spray to 1 part Tyrode solution). These dilutions were chosen according to Deitmer and Scheffler and other authors who used the same dilutions in similar in vitro experiments on CBF.6,9,12,13 The concentrations of isolated potassium sorbate and benzalkonium chloride in test series 1 and 2 were lower than the concentrations in the original nasal sprays (Table 2).

Neither Tyrode solution nor normal saline influence CBF. The fluids were kept at a constant temperature of 35°C in our system and could be changed at any time without interrupting the cell culture rinsing process. With this system, it was possible to create constant conditions such as temperature and osmolarity during measurements. A photometer was used to measure CBF.

Every measurement started with a run-in period of 20 minutes during rinsing with neutral Tyrode solution to measure the baseline CBF. After 20 minutes, the Tyrode solution was changed to test solution for 7 minutes, which was followed by a washout period with Tyrode solution for 30 minutes.

The preservative potassium sorbate did not influence CBF. No alteration of CBF was found at either 0.6-mg/mL or 0.12-mg/mL concentration (Figure 2). The preservative benzalkonium chloride showed a decrease in CBF at a concentration of 0.05 mg/mL and irreversible standstill at 0.1 mg/mL (Figure 3).

Budesonide nasal spray containing potassium sorbate showed no alteration of CBF at 10% dilution and minor reversible slowing of CBF at 50% percent dilution (Figure 4). The topical glucocorticoid mometasone furoate spray containing benzalkonium chloride as preservative showed a slight decrease in CBF at 10% dilution and irreversible standstill during rinsing with 50% dilution (Figure 5).

These results are comparable with previously published experiments on the topical steroid fluticasone propionate spray, also containing benzalkonium chloride, which caused reversible decrease of CBF after exposure at 10% percent dilution and complete standstill of cilia after rinsing with a 50% dilution (Figure 6).

In the present in vitro study, the preservative potassium sorbate did not show an influence on CBF (Figure 2). Benzalkonium chloride caused concentration-dependent ciliary inhibitory effect (Figure 3). The topical glucocorticoid nasal spray with aqueous budesonide containing potassium sorbate as preservative did not show a decrease of CBF at 10% dilution and showed minimal reversible decrease at 50% dilution (Figure 4). The topi-
cal glucocorticoid sprays with fluticasone propionate and mometasone furoate, both containing benzalkonium chloride as preservative, showed reversible decrease of CBF at 10% dilutions and complete irreversible standstill at 50% dilutions (Figures 5 and 6).

A highly significant correlation between CBF and mucus transport time has been observed by Duchateau et al. Previous in vitro experiments found inhibitory effects on cilia function through nasal decongestants, antifungal solutions, topical steroids, and the preservative benzalkonium chloride. In vivo investigations have found controversial results: Berg et al confirmed the presence of squamous cell metaplasia in an in vivo animal experiment after 21 days of exposure to a topical steroid containing benzalkonium chloride, while this effect was not observed after exposure to 0.9% sodium chloride or steroid without benzalkonium chloride. Boek et al used a gamma scintigraphy technique on 15 healthy volunteers whose noses were treated with technetium Tc 99–marked xylometazoline spray with ben-
zalkonium chloride. A nonsignificant mean reduction of mucociliary transport time was found. Storaas et al. investigated the effect of benzalkonium chloride on glandular secretion and nasal symptoms of healthy volunteers. During 10 days of repeated exposure to commonly used dosages of benzalkonium chloride, short-term glandular secretion or nasal pain occurred, but no exudative hyperresponsiveness or airway inflammation was found.

Long-term in vivo studies of topical corticosteroids with and without benzalkonium chloride have not confirmed damage to the ciliated cells of the nasal mucosa; placebo-controlled randomized studies have proven the efficacy and safety of topical steroid therapy for allergic rhinitis and nasal polyposis. Other than mild local adverse effects such as nasal dryness, irritation, or mild nasal bleeding, topical corticosteroids are well tolerated over years. According to Minshall et al., the percentage of ciliated epithelium tended to increase, and the cell metaplasia decreased, after 12 months of treatment with 200 µg of mometasone furoate once daily. After 1 and 5 years' administration of budesonide nasal aerosol, healthy nasal epithelium has been documented by histopathologic evaluation. Biopsy specimens of nasal mucosa did not show nasal mucosa atrophy after once-

Figure 4. For 20 minutes, ciliary beat frequency (CBF) was measured during rinsing with Tyrode solution. For 7 minutes, the cell cultures were exposed to budesonide spray at 10% dilution (test series 1) or 50% dilution (test series 2). Only short reversible slowing of CBF was measured during test series 2.

Figure 5. For 20 minutes, ciliary beat frequency (CBF) was measured during rinsing with Tyrode solution. For 7 minutes, the cell cultures were exposed to mometasone furoate spray at 10% dilution (test series 1) or 50% dilution (test series 2). Test series 1 caused short reversible slowing of CBF. After test series 2, complete and irreversible standstill of the cilia was observed.
daily treatment with 200 µg of fluticasone over 1 year but, instead, showed improved epithelial thickness under evaluation with light and electron microscopy. The diverse findings of in vitro and in vivo studies can be explained by the lack of the mucus layer in vitro, which protects the ciliated epithelium of the human nasal mucosa. In vivo measurements with saccharin or indigo blue tests are subjective; the technetium Tc 99m test is objective; but many factors, such as circadian and nasal rhythms, anatomic variations, potential infections, individual variations of mucus transport time, and the requirement of absolute constant breathing throughout the measurements, influence the results. Because of individual variations in probands, it is necessary to conduct a high number of in vivo experiments to get reliable results. In vitro experiments simulate in vivo conditions as closely as possible, but there will always be a difference from human physiologic characteristics. Nasal sprays are placed mainly in the region of the head of the inferior and middle turbinate when used in vivo. A possible influence on CBF is more likely to occur in vivo only in these parts of the nasal mucosa, where the concentration of the topical drug is high. Because of the lack of the mucus layer, in vitro cells are less resistant to toxic substances, and the ability for tissue repair is limited.

Nevertheless, in our opinion, in vitro experiments on CBF are important to evaluate the safety of topically administered drugs because only in vitro experiments guarantee constant conditions and exclude other factors, such as stress, hormone secretion, or inflammatory mediators, which may influence the sensitive ciliated cells. Osmolarity, pH, and temperature, which influence CBF, can be controlled under in vitro conditions. If there is a reduction in CBF found by different investigators in vitro under standardized conditions and exclusion of cofactors, it can be assumed that also in vivo an influence on CBF is present. The degree of this effect in vivo is difficult to quantify, but in the present study a clear difference between the tested substances was found. As Bachert discussed recently, one must be critical about the results of in vitro studies and should not overreact, as the German Federal Institute for Drugs and Medical Devices is now doing as it considers reducing the period of administration of benzalkonium chloride–containing nasal sprays to 5 to 7 days.

Because of the missing protective mucus layer, in vitro experiments cannot precisely duplicate in vivo conditions, but in our opinion they do help to objectify the influence of different substances on CBF. Substances that do not harm ciliated cells in vitro can be considered safe in vivo and in our opinion should be preferred by the pharmaceutical industry.

In conclusion, the preservative potassium sorbate and the topical nasal spray containing this preservative did not show a significant influence on CBF in vitro. This preservative can therefore be considered harmless to the motility of ciliated cells.

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