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The effect of different preparations of nasal decongestants on ciliary beat frequency in vitro*

T. Deitmer, R. Scheffler

ENT Department, Westfälische Wilhelms University, Münster, Germany

SUMMARY

Ciliated cells from the nasal mucosa of normal persons were collected in culture medium and exposed to either oxymetazoline without preservatives, oxymetazoline with preservatives, xylometazoline with preservatives, or sham (culture medium). There was a significant decrease in ciliary beat frequency only by the two drugs with preservatives after 20 min. After substitution of the test media with culture medium ciliary action did not recover in any group.

Key words: nasal mucosa, ciliary beat frequency, nasal decongestants, ciliated cells

INTRODUCTION

Nasal decongestants are one of the most widely used topical drugs, and their beneficial effect during common cold or sinusitis is well known. Little is known about the side effects on the function of ciliated cells, especially on the possible recovery of ciliary function after exposure to such decongestants.

Effects on mucociliary transport function can be evaluated by applying marker substances to the mucosa and measuring the transport velocity during drug administration (Simon et al., 1977; Sakethoo et al., 1978; Van den Donk et al., 1982; Holmberg and Bende, 1988; Hermens and Merkus, 1990; Philips et al., 1990). On the other hand, there is the possibility to assess ciliary beat frequency *in vitro* under drug action (Van den Donk et al., 1980a,b, 1982; Hybbinette and Mercke, 1982; Toremalm, 1985; Philips et al., 1990).

As it is well known that during a common cold nearly the whole layer of ciliated cells in the nose is lost due to the cytotoxic effect of the virus, it is of interest whether noxious effects on regenerating ciliary activity are detectable. In some studies (Dudley and Cherry, 1978; Van den Donk et al., 1982; Toremalm, 1985) detrimental deterioration of ciliary beat frequency by nose drops could be detected. After applying nasal decongestants we must be aware that local concentration on the mucosal surface will diminish rapidly by mucociliary transport or dilution by nasal hypersecretion. Profound insight into the pharmacokinetics of nasal decongestants in the normal or diseased nose is scanty. Principally it seems of interest to study the time course of ciliary function after administration of those solutions and diluting them again. For this reason we conducted the following study.

MATERIAL AND METHODS

We took nasal cytological biopsies from 15 healthy persons by scraping the nasal mucosa without local anaesthesia with a cytology brush of 2-mm diameter, as used in flexible bronchoscopy. The brush was agitated in a culture medium (Dulbecco's Modified Medium, Gibco) at 37°C to produce a cell suspension. This suspension was immediately transferred to the two wells of a culture chamber, each having a volume of 0.27 ml. The contents of these chambers could be changed by drainage and refilling through small injection needles (PENTZ-chamber, TCSC3, Fa. Heraeus).

By means of a phase-contrast microscope the vital cell preparation could be examined and the ciliary beat frequency of single cells measured by a built-in microphoto-oscillometer (Deitmer, 1989). We measured the ciliary beat frequency of the 10 most active cells for 10 subsequent seconds and stored the data in a computer file for further processing.

Twenty minutes after filling the chamber we obtained the first measurement as a baseline. Then we allowed sedimentation of the cells in the chamber into one part of the well by holding it upright for about 1 min. Thus, we were able to aspirate most of the culture medium without losing too many cells. By this approach, we could drain 0.22 ml and

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refill the well with the same amount of the test solution. After 20 min of incubation we took a second measurement. Next, we changed the solution in a similar manner, aspirating the test solution and exchanging it with culture medium to study the effects of a possible recovery by measuring after a further incubation time of 20 min.

As we had two-well chambers and needed enough time for changing the culture medium and measuring ciliary beat frequency, we limited our study to four groups: (1) 0.05% oxymetazoline without preservatives; (2) 0.05% oxymetazoline with preservatives (edetinacid, benzalkoniumchloride, sodium hydrogenphosphate, sodium hydroxide); (3) 0.1% xylometazoline with preservatives (edetinacid, benzalkoniumchloride, sodium hydrogenphosphate, sorbitol); and (4) culture medium (sham).

We diluted the test solution (1 volume of test solution and 9 volumes of culture medium). Thus, we imitated a dilution of the nose drops into about 1–1.5 ml of nasal secretions when applied *in vivo* in the way as recommended for use. The pH of the solutions was buffered to 7.4.

RESULTS

The mean values from 100 measurements (taken from 10 cells, and measured for 10 s in each preparation) from all 15 persons, the four groups of the study, and the three points of testing are presented in Figure 1.

For statistical evaluation of each preparation the value of the preceding test was taken into consideration by calculating the difference. Thus, we compared for each person the value after incubation with the test solution to the value after the first incubation in culture medium. Then, we considered the difference between the value after incubation with test solution and the value after redilution with culture medium, to study possible effects of recovery.

Applying the Tukey-test, as a test for analysis of variance between several groups at a significance of 0.05, there were the following significant differences: (1) Between the sham group and the group with oxymetazoline and preservatives; (2) between the sham group and the group with xylometazoline and preservatives; (3) between the groups with oxymetazoline with and without preservatives; and (4) between the group with oxymetazoline with preservatives and xylometazoline with preservatives.

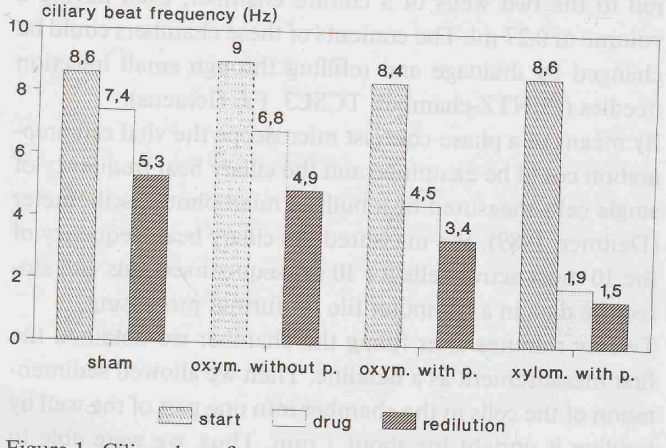

Figure 1. Ciliary beat frequency with different drugs (number of measurements: 15×100/column). Abbreviations: oxym.: oxymetazoline; p.: preservatives; xylom.: xylometazoline.

There were no differences between the sham group and the group with oxymetazoline without preservatives.

Looking at possible recovery effects after rinsing with culture medium we saw no rise of ciliary beat frequency. Of course the amount of previous deterioration of ciliary beat frequency by the test solution must be taken into account. The strongest fall in ciliary beat frequency was found in the sham group with a further ranking of oxymetazoline without preservatives, followed by oxymetazoline with preservatives, and xylometazoline with preservatives.

DISCUSSION

When evaluating the stability of our test preparation, a decrease in ciliary beat frequency in the sham group is obvious after 20 min and even more after 40 min. We think that manipulation of the cells during sedimentation, changing the solutions, and refilling the chamber is noxious for the ciliated cells, even when a temperature of 37°C is maintained. We added no antibiotics to our test design to exclude any pharmacological effects.

The incubation time of 20 min was suitable for our study design and can perhaps be compared to the *in vivo* situation. Provided an intact mucociliary transport system is present, such a time scale of pharmacological effects on the individual cells might be realistic; in case there is no mucociliary transport, but a hypersecretion from a common cold is, this time course may be true as well.

It would have been interesting to study an eventual recovery after periods longer than 20 min, but in the sham group the decrease was already obvious during the first 40 min, and a longer period of observation would need the use of antibiotics.

In previous tests we determined the suitable dilution of the test solutions. At dilutions of 2:8 or 4:6 (test solution volume to culture medium volume) we observed an immense decline in ciliary activity which did not allow detection of differences between the different groups, as there was a rapid deterioration of activity in all groups. That is the reason why we chose the somewhat arbitrary dilution of 1:9.

Noxious effects of oxymetazoline without preservatives on ciliary activity *in vitro* are not detectable, whereas there are effects of oxymetazoline and xylometazoline with preservatives. Van den Donk et al. (1981) also found a decrease in ciliary activity in a similar study design; in this study the effect was even more outspoken for xylometazoline than for oxymetazoline, both with preservatives. Toremalm (1985) reported a decrease of ciliary beat frequency by oxymetazoline without preservatives during 60 min and found a rapid arrest of ciliary activity when benzalkonium was added. Hybbinette and Mercke (1982) registered the ciliary beat frequency *in vivo* in a rabbit model and applied test solutions intra-arterially. In this study there

was a slowing of mucus wave frequency by action of phenylephrin and oxymetazoline, both without preservatives. Philips et al. (1990), on the other hand, found a dose-dependent increase of ciliary activity by phenylephrin at a concentration of up to 0.01% and a rapid decay at higher concentrations. Dudley and Cherry (1978) studied ciliated cells for 5 days and took the number of surviving cells as a parameter for drug effects. They saw longer survival times with xylometazoline than with oxymetazoline. Van den Donk et al. (1980b) conducted a study especially aimed at the effects of preservatives and found that all preservatives used were noxious. The effect of pH was negligible when held between 7 and 10 (Van den Donk et al., 1980a).

There are further studies on the pharmacology of nasal decongestants on the mucociliary system, measuring the transport time of marker substances. Whereas Holmberg and Bende (1988) found no changes after 1-week use of phenylpropanolamin, Saketkhoo et al. (1978) saw a significant acceleration during exposure to phenylephrine or tetrahydrozoline. The acceleration of mucociliary transport by phenylephrine could not be confirmed by Philips et al. (1990).

A testing of mucociliary transport with substances used in our study was only done with xylometazoline. An acceleration of transport was detected in two studies and seemed significant in the study of Simon et al. (1977), but not significant in the study of Van den Donk et al. (1982). As already mentioned the *in vivo* testing of nasal decongestants concomittantly affects the patency of the nose, and this may enhance mucociliary transport as well (Deitmer and Erwig, 1986). It is still unknown whether the observed acceleration of mucociliary transport *in vivo* by nasal decongestants is an effect on cilia or mediated by nasal patency and airflow.

In conclusion, it must be recommended to use nasal decongestants without preservatives or to develop preservatives that are not noxious for the cilia, especially since there seems to be no recovery of ciliary motility after rinsing of nasal decongestants in an *in vitro* model.

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Prof.Dr. med Th. Deitmer
Univ.-HNO-Klinik Münster
Kardinal von Galen Ring 10
D-4400 Münster
Germany