

In vitro effect of beclomethasone dipropionate and flunisolide on the mobility of human nasal cilia

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Human nasal cilia were perfused with aqueous solutions of two corticosteroid aerosols, beclomethasone dipropionate (BDP) and flunisolide, with the main preservative of flunisolide, propylene glycol, and with placebo. The concentrations used were BDP 0.1 mg/ml, 0.05 mg/ml, 0.005 mg/ml and 0.0005 mg/ml, flunisolide 0.25 mg/ml, 0.05 mg/ml, 0.025 mg/ml and 0.00025 mg/ml, and propylene glycol 20 mg/ml and 200 mg/ml. A dose-related decrease in ciliary beating frequency (CBF) was seen after perfusion with both BDP and flunisolide as well as propylene glycol. The decrease in CBF following perfusion with propylene glycol was partially reversible upon re-perfusion with medium alone, whereas the decrease seen after BDP and flunisolide was irreversible. Although previous studies have shown no adverse effect on the mucous membrane except for the areas hit by the impact of the sprays, our results suggest that caution should be taken when the dose and/or the length of treatment is considered, and that the effect of administration of these drugs on CBF *in vivo* needs to be investigated.

Key words: beclomethasone dipropionate; ciliary motility; flunisolide; propylene glycol; rhinitis.

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CLINICAL ASPECTS

The concentrations of either beclomethasone dipropionate (BDP) and flunisolide in the nasal secretion after nasal administration are not known but may be very high in the areas hit by the impact of the spray. However, no adverse effect on the mucous membrane has yet been seen. This study shows that *in vitro* perfusion with concentrations as low as 0.005 mg/ml BDP or 0.025 mg/ml flunisolide are capable of decreasing the ciliary beating frequency (CBF). In normals a decrease might not be serious but many of the patients who are treated with steroids, already before treatment have an impaired mucociliary clearance, and it is therefore of importance not to slow down the CBF if it can be avoided. No specific dosis can be recommended, but *in vivo* investigations on the effect of administration of these drugs on CBF are warranted.

The corticosteroid aerosol beclomethasone dipropionate (BPD) was introduced on the European market in the 1970s for the treatment of chronic rhinitis, and since 1978 flunisolide has also been used extensively.

Investigations have shown a beneficial clinical effect of both BPD (10, 12, 13, 14) and flunisolide (3, 18, 24, 28), and several comparative studies have concluded that the drugs are equally effective (1, 10, 25, 26).

In the skin, epidermal atrophy has been seen

by light microscopy after a few weeks of continuous topical treatment with potent corticosteroids (9), and several studies have been performed to investigate whether a similar effect could also be seen, either by light microscopy or by electron microscopy, in the respiratory tract after treatment with topical steroids in bronchi and nose (2, 5, 11, 16, 18, 22, 23, 27). In none of these studies was atrophy found in the specimens investigated. No decrease in the number of cilia was seen by scanning electron micro-

scopy (15). In fact, one group (5) found a progressive reappearance of normal cilia and microvilli on the columnar epithelial cells after 4–8 weeks treatment with BDP 200 μ g daily in each nostril. However, Pedersen et al. (20) found by direct inspection of the mucous membrane that the areas which were hit by the impact of the spray, i.e. the anterior part of the nasal septum, the tip of the inferior and middle turbinate, and the anterior side of a polyp, showed some mucosal dryness and a single crust was occasionally observed.

It has recently been shown that the ciliary beating frequency (CBF) is the main factor in nasal mucociliary clearance in healthy volunteers (4). Since topical steroids are propelled directly on to the nasal mucosa we found it relevant to investigate the direct effect of BDP and flunisolide on the ciliary function, using a microphoto-oscillographic *in vitro* method (19). We also investigated the main preservative in flunisolide, propylene glycol, since hyperosmolality has been seen as a result of transdermal absorption of this (6, 7), and since hyperosmolality has been found to decrease the CBF (29).

MATERIAL AND METHODS

Beclomethasone dipropionate (BDP), 0.5 mg/ml (Glaxo), was used concentrated and diluted with buffered Krebs Ringer solution enriched with glucose 5 mM/l (KRG) to give final concentrations in the perfusion solutions of 0.1 mg/l, 0.05 mg/ml, 0.005 mg/ml and 0.0005 mg/ml.

Flunisolide, 0.25 mg/ml (Syntex), was used concentrated and diluted with KRG to concentrations 0.05 mg/ml, 0.025 mg/ml and 0.00025 mg/ml.

Both BDP and flunisolide were aqueous solutions delivered from metered-dose pumps and therefore suitable for perfusion.

Propylene glycol was used in concentrations 200 mg/ml which is the concentration used in undiluted flunisolide (0.25 mg/ml) and 20 mg/ml.

KRG was used as placebo.

In all preparations the pH was adjusted to

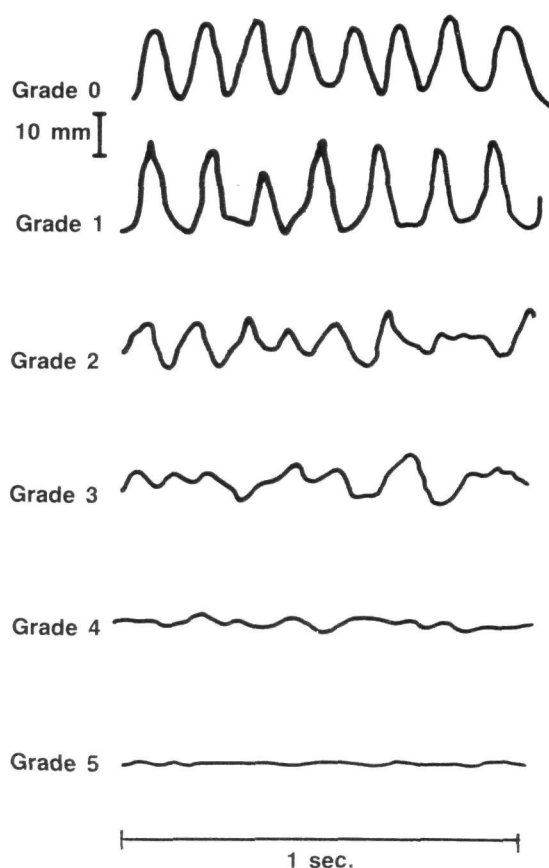


Fig. 1. Microphoto-oscillographic registration of ciliary motility on single cells. Examples of different grades of asynchrony from 0 (min. = complete synchrony) to 5 (max. = complete asynchrony) (26)

approximately 7.20 checked by routine measurements with a pH-meter (Radiometer, Copenhagen).

Cells from the mucous membrane of the inferior turbinate in the middle part of the nasal cavity were obtained from healthy volunteers by gentle scraping, using a small sharp spoon. The cells were placed in KRG at room temperature before use in the perfusion assays. Since only one perfusion could be done at a time the order of succession was changed regularly so as to compensate for any effect of storage.

Before measurements the ciliated cells were transferred to a Dworak-Stotler culture chamber with an inner diameter of 2 cm and a height of 40 μ m which produced a monolayer arrangement of the cells while still allowing free mobility of the cilia. The chamber was connected to an automated pump delivering 12 ml/h which gives a complete change of the fluid in the chamber in approximately 5 s.

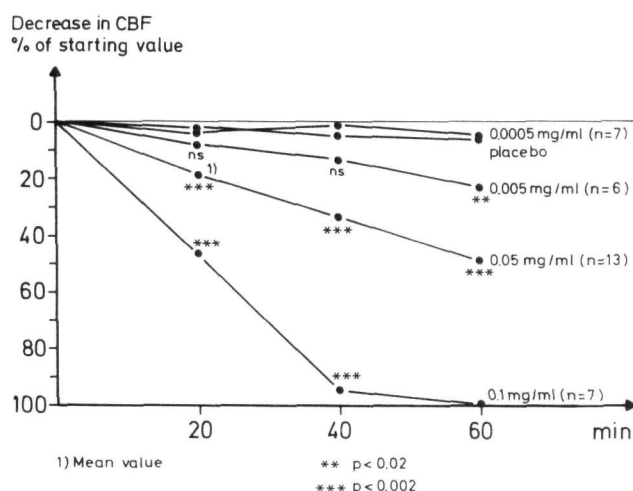


Fig. 2. Effect of perfusion with beclomethasone dipropionate on the human ciliary beating frequency (CBF) *in vitro*

Following a run-in period with KRG perfusion for 15 min, the perfusion with test solution, which was blinded to the investigator, was started. The temperature in the chamber was kept constant at approximately 26°C by means of the thermostat-controlled air stream incubator.

Recording of CBF and ciliary beating pattern was done by an anophtral phase contrast microscope (Zetopan, Reichert, Austria) equipped with a microphotometer which transforms the interference of light caused by the ciliary beating to a curve on a mingograph which depicts the frequency as well as the beating pattern (degree of synchrony between individual cilia). Synchronous ciliary beating results in almost completely uniform oscillations, whereas asynchrony between cilia from the same cell causes an irregular oscillogram. The degree of irregularity was graded as shown in Fig. 1 (21).

Four cells situated singly in order to ensure optimal exposure to the perfused substance were chosen. The beating frequency and pattern was measured during continuous perfusion every 20 min for 1 h for each of the four cells. Results were expressed as mean values of the four individual cells at each time of perfusion with each of the test solutions. If a test solution caused total cessation of ciliary movements, the specimen was reperfused with KRG to see if there was any reversibility.

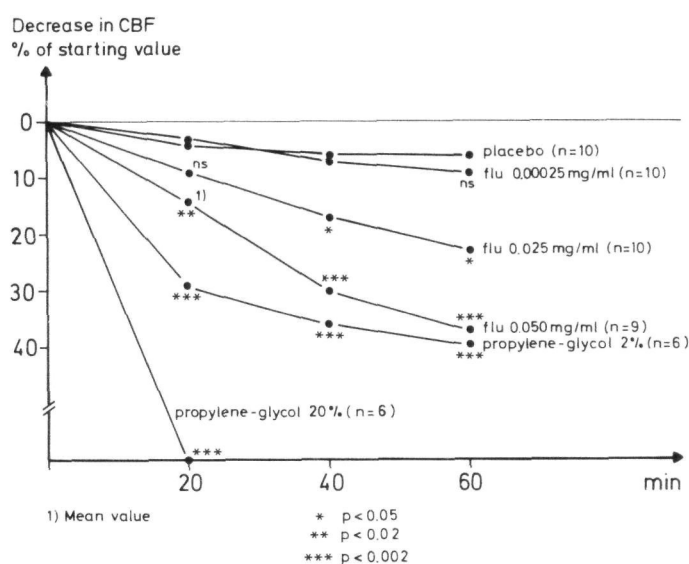


Fig. 3. Effect of perfusion with flunisolide (flu) and propylene glycol on the human ciliary beating frequency (CBF) *in vitro*

Statistical methods

The Mann-Whitney U test was used with two-sided alternatives.

RESULTS

The CBF after perfusion with different concentrations of BDP compared with placebo is shown in Fig. 2. The results are given in per cent of starting values. The number of investigations are given on the figure. Four cells were measured in each investigation (see Material and Methods). No effect was seen when the cilia were perfused with 0.0005 mg/ml BDP but a significant effect was seen after 1 h perfusion with 0.005 mg/ml, and the two strongest solutions showed a significant decrease already after 20 min. A clear dose-response effect was thus seen. BDP at 0.5 mg/ml caused complete cessation of ciliary beating after approximately 15 min, and at 0.1 mg/ml after 60 min. Reperfusion with KRG for 15 min starting immediately after cessation of ciliary beating did not show any reversibility at either of these concentrations.

In Fig. 3 the results from perfusion with flunisolide are shown. As was the case with BPD a clear dose-dependent depression of CBF was seen, and the effect was found to be signifi-

cant already after 20 min at a concentration of flunisolide of 0.05 mg/ml. No decrease was seen in the concentration, 0.00025 mg/ml. Perfusion with flunisolide (0.25 mg/ml) caused complete cessation after less than 10 min. When perfused with KRG afterwards most of the cells showed no reversibility; however, a very slow but synchronous beating was seen in a few after 15 min.

Perfusion with 20 mg/ml propylene glycol, which is the concentration in flunisolide 0.025 mg/ml, showed a clear decrease in CBF which was significant after 20 min, and 200 mg/ml propylene glycol, the concentration in the flunisolide spray, caused complete cessation in less than 20 min. Already 7 min after perfusion with KRG ciliary activity was seen again in all the cells, with a mean value of 82 % after 40 min ($n = 6$).

The degree of synchronicity within the single cell was within the normal range during all the measurements (grade 0–1) (21). The drugs thus only affected the CBF.

DISCUSSION

The dosage normally recommended when treating rhinitis is BDP 200 µg two times a day in each nostril or flunisolide 100 µg two times a day. To my knowledge no investigation of concentration of either BDP or flunisolide in the nasal secretion or the mucus has been made. The areas hit by the impact of the spray, i.e. the anterior part of the nasal septum, the tip of the inferior and middle turbinate and the anterior side of a polyp would, however, be expected to obtain rather high concentrations, which may result in decreased ciliary activity. However, the effect of these drugs on the CBF has never been studied.

Both drugs showed a clear dose-dependent depression of the CBF. Perfusion with flunisolide 0.25 mg/ml, which contains propylene glycol 20 %, caused complete cessation after less than 10 min. Propylene glycol 20 % alone had the same effect after approximately 15 min. Perfusion with propylene glycol 2 % showed a marked decrease after 40 min, whilst flunisolide 0.025 mg/ml, containing propylene glycol 2 %, only showed a 20 % decrease after 1 h. This could suggest that

the decrease seen after perfusion with flunisolide was due to its content of the preservative propylene glycol. If, however, this is the case it is not easy to understand why no reversibility was seen after perfusion with flunisolide since partial reversibility was seen after perfusion with propylene glycol alone.

The average nasal secretion under normal conditions is not known but can be as much as 10 ml/h when stimulated maximally (17). The nasal mucociliary clearance has recently been measured by Golhar (8) who found a nearly two-fold increase in time when the patients suffered from atopic rhinitis, vaso-motor rhinitis or early state of chronic maxillary sinusitis. In patients with late chronic maxillary sinusitis, bronchiectasis, or Kartagener's syndrome, he found a static mucociliary clearance. The difficulty in measuring the nasal secretion and the variety found in mucociliary clearance rates makes any calculations of the local concentrations following standard doses of nasally administered steroids very difficult. However, the many investigations which show no adverse effect on the mucous membrane in the nose or the respiratory tract when looked at either by eye, light microscope or electron microscope (2, 5, 11, 16, 18, 22, 23, 27) indicate that decreased CBF is not a major problem when treating with BPD or flunisolide aerosols. This could be due to either the protective property of the mucus and/or the rapid absorption and metabolism of the drugs (12, 18). Non-published data from this laboratory shows the CBF in normals at 26°C to be between 8.5 and 12.3 Hz. A decrease of 40 % might not be of clinical relevance to normal persons, but in patients with already impaired mucociliary clearance (8) the steroid concentration might be higher and work over a longer period, and in these cases a maximal obtainable CBF must be of importance. Further investigations on the *in vivo* effect of BDP and flunisolide on CBF in such patients should therefore be performed.

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