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The effect of nasal drug formulations on ciliary beating in vitro

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Abstract

In order to estimate the potential of nasal drug formulations to influence ciliary beating, ciliary beat frequency (CBF) measurements were performed in vitro, using chicken embryo trachea and a photo-electric registration device. The effects of nasal prescription and non-prescription drug formulations were studied and compared with a number of investigational nasal drug formulations containing estradiol, dihydroergotamine mesylate and salmon calcitonin. The influence of all formulations on CBF was related to the effects of formulation additives such as preservatives and nasal absorption enhancers on the ciliated tissue. For almost all nasal drug formulations, the preservatives used in the formulations (e.g. benzalkonium chloride, chlorobutanol) play a decisive role in the observed ciliostatic effects. Methylated β-cyclodextrins, used as nasal absorption enhancers/solubilizers in the investigational formulations, appeared to be relatively non-toxic for the ciliated tissue, having similar effects on CBF as physiological saline. After dilution five times, most drug formulations studied showed a moderate ciliostatic effect, but marked differences could still be detected. The present study demonstrates that CBF measurements in vitro are a valuable tool in the design of safe nasal drug formulations.

Keywords: Ciliary beat frequency; Nasal (non-)prescription drug formulations; Preservatives; Absorption enhancers

1. Introduction

Nasal drug formulations for local use are widely used as they are mostly 'over the counter' drugs. They are indicated for frequently occurring diseases such as the common cold and hayfever. There is an increased number of drugs which are administered nasally for systemic use, since the nasal mucosa is an effective site for drug absorption, as the surface of the nasal mucosa is large and provided with blood vessels. With nasal absorption the first-pass effect and the gastrointestinal degradation of drugs can be avoided (Chien et al., 1989).

In particular the systemic delivery of peptide and protein drugs — such as insulin (Illum and Davis, 1992; Jacobs et al., 1993), calcitonin (Overgaard et al., 1991; Schipper et al., 1995), desmopressin (Critchley et al., 1994) and buserelin (Heinrichs et al., 1994) — by nasal administration is currently an area of research and drug development. The poor bioavailability of many of these compounds can be improved substantially by the

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use of nasal absorption enhancers. The acceptability of these enhancers is not only dependent on their absorption promoting effect, but also on their safety profile regarding systemic effects and also local adverse effects (Lee et al., 1991; Merkus et al., 1993).

Nasal drug formulations should not interfere with the self-cleaning capacity of the nose, effectuated by the ciliary epithelium. The coordinated beating of the cilia results in the movement of the upper mucus layer towards the nasopharynx. The combined action of cilia and mucus layer is called mucociliary clearance, in which the ciliary movement plays an important role (Andersen and Proctor, 1983; Lansley, 1993). It is, therefore, relevant to study the effect of drugs and pharmaceutical excipients for nasal drug delivery on ciliary beat frequency (CBF), because it helps to design formulations with a minimal or acceptable toxicity profile. The measurement of the CBF in vitro is very accurate and reproducible (Van de Donk et al., 1982; Batts et al., 1990; Ingels et al., 1991; Rusznak et al., 1994), but because of the use of excised tissue without the protective effect of the mucus barrier, it is not possible to make predictions regarding the effects of chronic use of a formulation on mucociliary clearance in vivo. Nevertheless, it gives valuable information on the ciliotoxic potency of a nasal drug or excipient.

In order to compare the potential of drugs, formulation additives such as preservatives and nasal absorption enhancers to influence ciliary beating, in this paper the effect of these constituents and formulations on CBF in vitro is presented.

2. Materials and methods

2.1. Materials

Benzalkonium chloride (BAC; U.S.P. quality) and D-mannitol were obtained from Brocacef (Maarssen, The Netherlands). Chlorobutanol was from Sigma-Chemie (Deisenhofen, Germany). Two methylated cyclodextrins with a degree of substitution near 2 were used: dimethyl-β-cyclodextrin (DMβCD) (D.S. 2.0) and randomly methylated β-cyclodextrin (MβCD) (D.S. 1.8), from AVEBE (Foxhol, The Netherlands) and Wacker (Burghausen, Germany), respectively. Estradiol (E_2) was acquired from Diosynth (Oss, The Netherlands). Dihydroergotamine methanesulfonate (DHE) was from Sigma (St. Louis, MO, USA), and salmon calcitonin (sCT, 5044 IU/mg) from UCB Bioproducts (Braine-l'Alleud, Belgium). All other chemicals used were of analytical grade.

2.2. Nasal drug formulations

2.2.1. Non-prescription drug formulations (Table 1)

Afrin was from Schering (Kenilworth, USA), Dristan from Whitehall Laboratories (New York, USA), Nasivin from Merck (Darmstadt, Germany), Nostrilla from Boehringer Ingelheim (Ridgefield, USA), Otrivin from Ciba-Geigy (Basle, Switzerland), Rinileen from VSM Medicals (Alkmaar, The Netherlands), and Sinex from Richardson-Vicks (Shelton, USA).

2.2.2. Prescription drug formulations (Table 1)

Miacalcic, a nasal spray formulation of salmon calcitonin, was obtained from LPB Instituto Farmaceutico S.P.A. (Milan, Italy). Minrin, a nasal desmopressin solution, was from Ferring (Malmö, Sweden).

2.2.3. Investigational nasal formulations

The two nasal estradiol (E_2) formulations contained estradiol in concentrations of 2 and 4 mg/ml, respectively in a MβCD (2 and 4% w/v, respectively) inclusion complex in physiological saline with 0.01% (w/v) benzalkonium chloride. Dihydroergotamine methanesulfonate (DHE) nasal preparation consisted of 4 mg DHE per ml deionized water with 4% (w/v) MβCD and 5% (w/v) D-mannitol. Nasal salmon calcitonin (sCT) formulation contained 100 IU of sCT and 2% (w/v) DMβCD per ml physiological saline.

2.3. Locke-Ringer solution

Locke-Ringer (LR) is an isotonic solution of the following composition per litre of water:

Table 1
Composition of non-prescription and prescription drug formulations tested

Product	Preservative	Conc.(% w/v)	Drug	Conc.(% w/v)	Other additives	BAC[a] (% w/v) measured	pH measured
A	Not given	Not given	Oxymetazoline	0.05	Buffer	0.01	7.0
B	BAC[a]	Not given	Xylometazoline	0.1	Buffer	0.01	6.3
C	Thimerosal	0.001	Phenylephrine	0.5	Campher Eucalyptol	–	7.0
	Cetylpyridinium chloride	0.04			Menthol Tyloxapol Buffer		
D	BAC	Not given	Oxymetazoline	0.05	Campher Eucalyptol	0.01	5.0
	Phenylmercuric acetate	0.002			Menthol Sorbitol Glycine		
E	BAC	0.02	Oxymetazoline	0.05	Sorbitol Glycine	0.02	5.9
F	BAC	0.02	Xylometazoline	0.05	Hydroxypropyl methyl cellulose	0.02	6.2
	Thimerosal	0.002			Buffer		
G	BAC	0.01	Homeopathic			0.01	5.0
H	BAC	0.01	Calcitonin	550 IU/ml	NaCl	0.01	5.0
I	Chlorobutanol	0.5	Desmopressin	0.01	NaCl	–	4.6

[a]BAC, benzalkonium chloride.
Registered Trade Marks: A, Nasivin; B, Otrivin; C, Sinex; D, Afrin; E, Nostrilla; F, Dristan; G, Rinileen; H, Miacalcic; I, Minrin.

NaCl, 7.72 g (132 mmol); KCl, 0.42 g (5.63 mmol); $CaCl_2 \cdot 2H_2O$, 0.16 g (1.24 mmol); $NaHCO_3$, 0.15 g (1.79 mmol); glucose·H_2O, 1.00 g (5.05 mmol). Locke-Ringer solution was prepared using Millipore-deionized water, and the solution was subsequently sterilized for 20 min at 120°C.

2.4. Ciliary beat frequency measurements

Ciliary beat frequency (CBF) measurements were performed on the ciliated epithelium of isolated chicken embryo trachea as described previously (Van de Donk et al., 1980). Briefly, the chicken embryo trachea was dissected from the embryo, and sliced into small rings of approximately 1 mm thickness. The trachea slices were placed in stainless steel supporting rings, and were allowed to recover for 30 min in Locke-Ringer solution. Then, the tissue samples were put in a well containing 1.0 ml of the test solution, and placed under an Olympus BH-2 light microscope. The microscope table was connected with a thermostat to maintain a temperature of 33°C. The CBF was subsequently monitored using a photo-electric registration device. A light beam was transmitted through the moving cilia and, after magnification by the microscope, the flickering light was projected to a photocell. The electrical signal generated by this photocell was visualized with a computer monitor. The frequency of the signal was calculated electronically by a Fast Fourier Transform algorithm and displayed as a frequency distribution. The CBF was measured every 5–10 min during a period of 1 h. Data were calculated as the relative frequency of the initial frequency measured in Locke-Ringer solution at the start of the experiment, the latter being expressed as 100%.

Table 2
The effect of nasal drug formulations on ciliary beat frequency of chicken embryo trachea in vitro

Preparation	Time (min)								
	5	10	15	20	30	40	50	60	n
Controls									
Locke-Ringer (blank)		98 ± 3		95 ± 3	100 ± 2	100 ± 4	103 ± 3	101 ± 4	8
NaCl 0.9%		88 ± 6		72 ± 4	62 ± 4	56 ± 5	53 ± 6	41 ± 5	10
(Non-)prescription formulations									
C	0[a]	0[a]		0[a]	0[a]	0[a]	0[a]	0[a]	6
D	0[a]	0[a]		0[a]	0[a]	0[a]	0[a]	0[a]	6
I	0[a]	62 ± 7[a]		71 ± 10[a]	75 ± 10	76 ± 15[a]	78 ± 14[a]	75 ± 13[a]	6
E	16 ± 7	0[a]		0[a]	0[a]	0[a]	0[a]	0[a]	6
A	32 ± 7	2 ± 2	0[a]	13 ± 5[a]	14 ± 5[a]	21 ± 9[a]	26 ± 10[a]	27 ± 10[a]	10
F	38 ± 9	1 ± 1	0[a]	18 ± 8[a]	27 ± 12[a]	22 ± 11[a]	27 ± 16[a]	25 ± 11[a]	5
B	51 ± 9	25 ± 3	4 ± 2	0[a]	6 ± 4[a]	12 ± 6[a]	22 ± 9[a]	22 ± 11[a]	10
H		49 ± 9		27 ± 5	5 ± 2	6 ± 3	3 ± 2	0	8
G		36 ± 5		36 ± 3	24 ± 4	14 ± 5	8 ± 5	5 ± 3	7
Investigational formulations									
sCT		66 ± 5		48 ± 5	34 ± 9	21 ± 8	8 ± 6	4 ± 11	6
E_2 (2 mg/ml)		50 ± 6		38 ± 6	25 ± 4	18 ± 4	15 ± 5	8 ± 3	8
E_2 (4 mg/ml)		36 ± 3		25 ± 4	14 ± 3	15 ± 3	10 ± 3	3 ± 2	8
DHE	49 ± 7	19 ± 9	2 ± 2	0[a]	5 ± 3[a]	5 ± 3[a]	5 ± 3[a]	5 ± 3[a]	6
Enhancers and preservatives									
MßCD (2%)		66 ± 5		73 ± 9	71 ± 7	76 ± 5	76 ± 6	70 ± 5	6
MßCD (4%)		77 ± 3		61 ± 3	52 ± 5	33 ± 5	27 ± 4	24 ± 7	8
DMßCD (2%)		75 ± 4		74 ± 7	70 ± 5	66 ± 6	59 ± 8	51 ± 8	7
BAC (0.01%)		51 ± 8		49 ± 10	41 ± 10	18 ± 5	8 ± 3	4 ± 2	8
BAC (0.02%)		63 ± 9		24 ± 7	5 ± 3	0	0	0	11
Chlorobutanol (0.5%)	0[a]	43 ± 11[a]		47 ± 8[a]	57 ± 5[a]	69 ± 6[a]	64 ± 3[a]	76 ± 8[a]	6

All values are presented as percentages of the initial frequencies (t_o = 100%) and are mean ± S.E.M. of the indicated number (n) of experiments.
[a]Tissue was replaced in pure Locke-Ringer after CBF was zero (reversibility testing).
Registered Trade Marks: A, Nasivin; B, Otrivin; C, Sinex; D, Afrin; E, Nostrilla; F, Dristan; G, Rinileen; H, Miacalcic; I, Minrin. BAC, benzalkonium chloride; DHE, dihydroergotamine methanesulfonate; DMßCD, dimethyl-ß-cyclodextrin; E_2, estradiol; MßCD, methylated ß-cyclodextrin; sCT, salmon calcitonin.

2.5. Test formulations

All nasal formulations were studied in undiluted form (Table 2). The additives used in the investigational nasal formulations were also measured for their effect on ciliary beating, after dissolving these compounds in Locke-Ringer: MβCD in concentrations of 2 and 4% (w/v), DMβCD in a concentration of 2% (w/v), the preservative benzalkonium chloride in concentrations of 0.01 and 0.02% (w/v) and the preservative chlorobutanol in a concentration of 0.5% (w/v). Control experiments (blank) were performed in pure Locke-Ringer.

A number of nasal preparations was also measured for their effect on CBF after a five-times dilution in Locke-Ringer solution (Table 3). For all formulations which resulted in complete ciliostasis (i.e. CBF 0%) within 25 min, the reversibility of the CBF was investigated. At time points whereupon CBF reached values of zero, the trachea slices were washed by shaking them vigorously in a tube with 3 ml Locke-Ringer. The slices were subsequently replaced in pure Locke-Ringer solution, and CBF was measured again every 5–10 min until 60 min after the start of the experiments.

Table 3
The effect of nasal drug formulations (five-times diluted in Locke-Ringer) on ciliary beat frequency of chicken embryo trachea in vitro

(Non-)prescription formulations	Time (min)							
	5	10	15	20	30	40	50	60
C	14 ± 6	7 ± 4	0[a]	7 ± 3[a]	21 ± 10[a]	25 ± 13[a]	19 ± 6[a]	17 ± 6[a]
D	13 ± 1	5 ± 3	2 ± 2	0[a]	17 ± 5[a]	29 ± 8[a]	29 ± 5[a]	41 ± 9[a]
I	35 ± 11	18 ± 8	10 ± 4	11 ± 4	5 ± 4	5 ± 3	9 ± 7	7 ± 5
F		59 ± 7		39 ± 7	37 ± 8	39 ± 10	32 ± 6	26 ± 9
E		95 ± 7		51 ± 6	61 ± 9	54 ± 11	56 ± 7	36 ± 8
G		81 ± 4		76 ± 3	62 ± 6	57 ± 5	45 ± 5	39 ± 5
B		74 ± 6		66 ± 7	62 ± 7	52 ± 8	45 ± 8	50 ± 7
A		76 ± 5		74 ± 7	61 ± 9	55 ± 10	57 ± 7	54 ± 11

All values are presented as percentages of the initial frequencies (t_0 = 100%) and are mean ± S.E.M. of five or six experiments.
[a]Tissue was replaced in pure Locke-Ringer after CBF was zero (reversibility testing).
Registered Trade Marks: A, Nasivin; B, Otrivin; C, Sinex; D, Afrin; E, Nostrilla; F, Dristan; G, Rinileen; I, Minrin.

2.6. Analysis of benzalkonium chloride

Since the concentration of the preservative benzalkonium chloride was not mentioned for all nasal drug formulations studied, the amounts of benzalkonium chloride were measured by reversed-phase HPLC (Fan and Wall, 1993).

3. Results

The results of the effect of all nasal drug formulations studied in undiluted form on the CBF are given in Table 2, whereas the results obtained with a number of five-times diluted formulations in Locke-Ringer solution are depicted in Table 3.

3.1. Locke-Ringer and physiological saline

The CBF of the blank (pure Locke-Ringer solution) remained around 100% of the initial frequency during the 60 min of the experiments (Table 2; Fig. 1). Physiological saline (0.9% NaCl) decreased ciliary beating with time, leading to CBF values of 41 ± 5% of the initial values after 60 min of incubation.

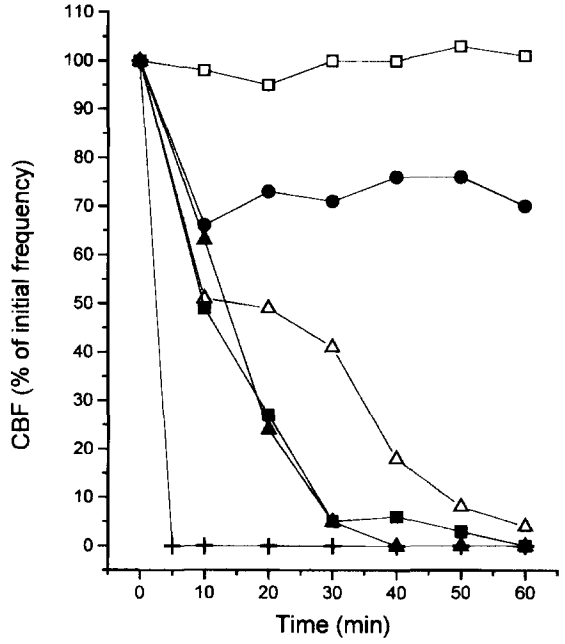

Fig. 1. The effect of representative formulations and excipients on ciliary beat frequency of chicken embryo trachea in vitro. □, Locke-Ringer; ●, methylated β-cyclodexrin 2%; △, benzalkonium chloride 0.01%; ▲, benzalkonium chloride 0.02%; ■, Miacalcic; +, Afrin. Data are the mean of 6–11 experiments.

3.2. Non-prescription nasal formulations

The formulations C (Sinex) and D (Afrin) appeared to be ciliotoxic, showing complete and irreversible ciliostasis within 5 min after exposure to the undiluted preparations (Table 2; Fig. 1). However, the effect of both formulations diluted in Locke-Ringer was less severe. C and D arrested the ciliary beating within 15 and 20 min, respectively, but after washing the ciliated tissue in pure Locke-Ringer the CBF recovered to mean values of 17% and 41% at $t = 60$ min, respectively (Table 3).

Formulations E (Nostrilla), A (Nasivin), F (Dristan) and B (Otrivin) in undiluted form reduced the ciliary beating to zero within 10–20 min (Table 2). Subsequent washing of the trachea tissue in Locke-Ringer solution showed partial recovery of the CBF for A, F and B to mean values of 27%, 25% and 22%, respectively, whereas the effect of undiluted E was irreversible. As apparent from Table 3, five-times dilution of E, A, F and B in Locke-Ringer resulted in milder effects on CBF than the undiluted formulations, and ciliary activity was still observed at 60 min after the start of the experiments.

The undiluted homeopathic formulation G (Rinileen) reduced the CBF in 60 min to 5 ± 3% of its initial frequency (Table 2). The effect of five-times diluted G on ciliary beating was mild, gradually decreasing the CBF to values of 39 ± 5% (Table 3).

3.3. Prescription nasal formulations

Formulation I (Minrin) in undiluted form led to complete ciliostasis within 5 min, but ciliary movement appeared to be reversible after rinsing the tissue with pure LR and CBF regained a value of 75 ± 13% at 60 min after the start of the experiment (Table 2). After five-times dilution of I in LR solution, the observed effect on CBF was much less severe (Table 3).

As evident from Table 2 and Fig. 1, undiluted formulation H (Miacalcic) decreased the CBF within 30 min to 5 ± 2% of its initial frequency.

3.4. Investigational nasal formulations

The salmon calcitonin (sCT) and the two estradiol (E_2) formulations studied gradually reduced the CBF to almost zero in 60 min (Table 2). The dihydroergotamine formulation (DHE) arrested the ciliary activity within 20 min. After washing the ciliated tissue with pure Locke-Ringer, the CBF showed only a tendency to recover (Table 2).

3.5. Other preparations and excipients

Exposure to the cyclodextrin derivative MβCD at final concentrations of 2 and 4% (w/v) in Locke-Ringer reduced CBF within 10 min to mean values of 66–77% of the initial frequencies. Thereafter, the CBF remained fairly constant up to 60 min for 2% MβCD (Table 2; Fig. 1), whereas the ciliary activity gradually decreased to mean values of 24% of the initial CBF for 4% MβCD. The effects of 2% DMβCD and 2% MβCD on ciliary beating were similar.

The effect of the preservative benzalkonium chloride in concentrations of 0.01 and 0.02% (w/v) on ciliary beating is also shown in Table 2 and Fig. 1. A concentration of 0.01% gradually decreased CBF to almost zero in 60 min, whereas a concentration of 0.02% stopped CBF within 40 min. The preservative chlorobutanol in a concentration of 0.5% (w/v) stopped ciliary movement within 5 min, but this ciliostasis appeared to be reversible after replacing the tissue in Locke-Ringer to 76 ± 8% of the initial frequency at 60 min after the start of the experiment.

4. Discussion

In the search for safe nasal drug formulations, measuring the effect of drugs and additives on the CBF is a quick and valuable approach. Since a good correlation has been found between the effects of nasal drugs, preservatives and absorption enhancers on the CBF of chicken trachea and human adenoid tissue, ciliated tissue from chicken tracheas is a suitable model for studying ciliostatic activity (Van de Donk et al., 1982; Merkus et al., 1993).

From the present results obtained with the non-prescription drug formulations, it is apparent that C and D have the strongest effect on CBF. Thimerosal 0.01% (used in C) and phenylmercuric acetate (used in D) were found to have a much stronger inhibitory effect on CBF than BAC 0.01% (Van de Donk et al., 1980). Thus, a major cause of this strong ciliostatic activity are the preservatives present in the formulation (Table 1). The constituents camphor, menthol and eucalyptol (present in both C and D) also play an additional role. By comparing two nasal formulations both containing ephedrine HCl 1% but one of them also a combination of menthol 0.015%, camphor 0.015% and eucalyptol 0.1%, it has been shown that the latter additives had a strong inhibitory effect on ciliary movement (Van de Donk et al., 1981; Su et al., 1993).

The non-prescription nasal formulations E, A, F and B arrested CBF within 10–20 min. Since α_2-adrenoceptor agonists such as xylometazoline and oxymetazoline decrease ciliary activity (Van de Donk et al., 1981; Cervin et al., 1988), the results found for these products are probably a combined effect of both drugs and preservatives. The ciliostasis appeared to be partially reversible for A, F and B, but not for E. Both E and F have 0.02% BAC as preservative and both E and A contain oxymetazoline (instead of xylometazoline) as active drug, so the only difference to explain the irreversibility of the CBF with E is the presence of sorbitol and glycine as additives in formulation E. The homeopathic non-prescription formulation G, containing 0.01% BAC, had an effect on CBF, quite similar to that found for BAC 0.01% alone (Table 1).

The two prescription formulations studied (H and I) are both nasal peptide formulations. Peptide drugs are macromolecular and hydrophilic compounds and have negligible ciliostatic potency. For instance, 1.0% (w/v) human insulin (Hermens et al., 1990) and salmon calcitonin (100 IU/ml; unpublished results), both in Locke-Ringer solution, had the same negligible effect on CBF as the control Locke-Ringer solution. Therefore, it is obvious that the effect of formulation H (Miacalcic) is similar to that of the preservative 0.01% BAC and that the profound but reversible ciliostatic activity of formulation I (Minrin) can be ascribed to the presence of the preservative 0.5% chlorobutanol.

For the investigational nasal drug formulations, methylated cyclodextrins were used as additives. In the sCT formulation DMβCD was included as a peptide absorption enhancer, whereas in the two estradiol and the DHE formulations MβCD was used as a solubilizer and absorption enhancer of these two lipophilic drugs. The effect of the sCT formulation on the ciliary beating can be interpreted as a combined effect of 0.9% NaCl and 2% DMβCD. The two estradiol formulations both contained 0.01% BAC and showed the same effect as this preservative alone (Table 2). From the results obtained with the DHE formulation, it is apparent that DHE itself has a strong reducing effect on CBF.

When comparing the influence of the various nasal prescription and non-prescription formulations on the CBF in vitro, it can be concluded that the preservatives in use play a decisive role. The effect on the CBF of the investigational nasal estradiol and calcitonin formulations were mild. In fact, the ciliostatic potential of 2% DMβCD and 2% MβCD, used as additives in the calcitonin and estradiol (2 mg/ml) formulation, respectively, appeared to be similar to that of 0.9% NaCl (Table 2).

In establishing the actual local toxicity of nasal drug formulations, measuring CBF in vitro is probably a too sensitive approach. In vivo experiments in rats, monkeys and in men showed no deleterious effects on nasal ciliated epithelium of corticosteroid formulations containing the preservative BAC in concentrations up to 0.02% (Ainge et al., 1994; Stanley et al., 1985). Since the healthy human nose has about 0.4 ml mucus (Stanley et al., 1985) and generally used nasal spray volumes are in the order of 0.1 ml, a five-times dilution of the nasal drug formulations may give a more realistic view in their ciliostatic potential. As evident from Table 3, most of the drug formulations studied have a moderate effect after five-times dilution, but marked differences can still be detected. The formulations C (Sinex), D (Afrin) and I (Minrin), especially, exhibited ciliostasis, also in diluted form. For acute and subchronic use, possi-

ble local toxicity is of minor importance, because the epithelial cells in the nasal mucosa are continuously replaced by cells that differentiate from basal stem cells at the basement membrane. In this way, ciliated tissue can be expected to recover before a subsequent nasal drug administration.

The in vitro CBF model has proven its predictive value in the past. For a large number of nasal absorption enhancing compounds such as surfactants, bile salts and fusidate derivatives, a good relationship (i.e. similarity in rank order) has been established between the morphological damage of the nasal epithelial membranes, the release of proteins and lipids in the nasal cavity, and the influence on the CBF in vitro (Merkus et al., 1993; Marttin et al., 1995). These studies also showed that the methylated β-cyclodextrins are relatively non-toxic solubilizers and absorption enhancers. From the results of this study it can be concluded that CBF measurements in vitro are a valuable tool in the design of safe nasal drug formulations.

References

Ainge, G., Bowles, J.A.K., Mc Cormick, S.G., Richards, D.H. and Scales, M.D.C., Lack of deleterious effects of corticosteroid sprays containing benzalkonium chloride on nasal ciliated epithelium in vivo results in laboratory animals. *Drug. Invest.*, 8 (1994) 127–133.

Andersen, I. and Proctor, D.F., Measurement of nasal mucociliary clearance. *Eur. J. Resp. Dis.*, 64 (1983) 37–40.

Batts, A.H., Marriott, C., Martin, P., Wood, C.F., and Bond, W., The effect of some preservatives used in nasal preparations on the mucus and ciliary components of mucociliary clearance. *J. Pharm. Pharmacol.*, 42 (1990) 145–151.

Cervin, A., Bende, M., Lindberg, S., Mercke, U. and Olsen, P., Relations between blood flow and mucociliary activity in the rabbit maxillary sinus. *Acta Otolaryngol.*, 105 (1988) 350–356.

Chien, Y.W., Su, K.S.E. and Chang, S.F., *Nasal Systemic Drug Delivery*. Marcel Dekker, New York, 1989.

Critchley, H., Davis, S.S., Farraj, F. and Illum, L., Nasal absorption of desmopressin in rats and sheep. Effect of a bioadhesive microsphere delivery system. *J. Pharm. Pharmacol.*, 46 (1994) 651–656.

Fan, T.Y. and Wall, G.M., Determination of benzalkonium chloride in ophthalmic solutions containing tyloxapol by solid-phase extraction and reversed-phase high-performance liquid chromatography. *J. Pharm. Sci.*, 82 (1993) 1172–1175.

Heinrichs, C., Craen, M., Vanderschueren-Lodeweyckx, M., Malvaux, P., Fawe, L. and Bourguignon, J.P., Variations in pituitary-gonadal suppression during intranasal buserelin and intramuscular depot-triptorelin therapy for central precocious puberty. *Acta Paediatr.*, 83 (1994) 627–633.

Hermens, W.A.J.J., Hooymans, P.M., Verhoef, J.C. and Merkus, F.W.H.M., Effects of absorption enhancers on human nasal tissue ciliary movement in vitro. *Pharm. Res.*, 7 (1990) 144–146.

Illum, L. and Davis, S.S., Intranasal insulin-clinical pharmacokinetics. *Clin. Pharmacokinet.*, 23 (1992) 30–41.

Ingels, K.J.A.O., Kortman, M.J.W., Nijziel, M.R., Graamans, K. and Huizing, E.H., Factors influencing ciliary beat measurements. *Rhinology*, 29 (1991) 17–26.

Jacobs, M.A.J.M., Schreuder, R.H., Jap-A-Joe, K., Nauta, J.J., Andersen, P.M. and Heine, R.J., The pharmacodynamics and activity of intranasally administered insulin in healthy male volunteers. *Diabetes*, 42 (1993) 1649–1655.

Lansley, A.B., Mucociliary clearance and drug delivery via the respiratory tract. *Adv. Drug. Deliv. Rev.*, 11 (1993) 299–327.

Lee, V.H.L., Yamamoto, A. and Kompella, U.B., Mucosal penetration enhancers for facilitation of peptide and protein drug absorption. *Crit. Rev. Ther. Drug. Carrier Syst.*, 8 (1991) 91–192.

Marttin, E., Verhoef, J.C., Romeijn, S.G. and Merkus, F.W.H.M., Effects of absorption enhancers on rat nasal epithelium in vivo: release of marker compounds in the nasal cavity. *Pharm. Res.*, 12 (1995) 1151–1157.

Merkus, F.W.H.M., Schipper, N.G.M., Hermens, W.A.J.J., Romeijn, S.G. and Verhoef, J.C., Absorption enhancers in nasal drug delivery: efficacy and safety. *J. Controlled Release*, 24 (1993) 201–208.

Overgaard, K., Angusdei, D., Hansen, M.A., Maioli, E., Christiansen, C. and Gennari, C., Dose-response bioactivity and bioavailability of salmon calcitonin in premenopausal and postmenopausal women. *J. Clin. Endocrinol. Metab.*, 72 (1991) 344–349.

Rusznak, C., Devalia, J.L., Lozewicz, S. and Davies, R.J., The assessment of nasal mucociliary clearance and the effect of drugs. *Resp. Med.*, 88 (1994) 89–101.

Schipper, N.G.M., Verhoef, J.C., Romeijn, S.G. and Merkus, F.W.H.M., Methylated ß-cyclodextrins are able to improve the nasal absorption of salmon calcitonin. *Calcif. Tissue Int.*, 56 (1995) 280–282.

Stanley, P.J., Griffin, W.M., Wilson, R., Greenstone, M.A., Mackay, I.S. and Cole, P.J., Effect of betamethasone and betamethasone with neomycin nasal drops on human nasal mucociliary clearance and ciliary beat frequency. *Thorax*, 40 (1985) 607–612.

Su, X.Y., Li Wan Po, A. and Millership, J.S., Ciliotoxicity of intranasal formulations: menthol enantiomers. *Chirality*, 5 (1993) 58–60.

Van de Donk, H.J.M., Muller-Plantema, I.P., Zuidema, J. and Merkus, F.W.H.M., The effects of preservatives on the ciliary beat frequency of chicken embryo tracheas. *Rhinology*, 18 (1980) 119–133.

Van de Donk, H.J.M., Zuidema, J. and Merkus, F.W.H.M., The effect of nasal drops on the ciliary beat frequency of chicken embryo tracheas. *Rhinology*, 19 (1981) 215–230.

Van de Donk, H.J.M., Zuidema, J. and Merkus, F.W.H.M., Correlation between the sensitivity of the ciliary beat frequency of human adenoid tissue and chicken embryo tracheas for some drugs. *Rhinology*, 20 (1982) 81–87.