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Report

Effects of Absorption Enhancers on Human Nasal Tissue Ciliary Movement *in Vitro*

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Sodium taurodihydrofusidate (STDHF) is one of the most promising absorption enhancers for nasal delivery of peptide drugs. Drugs and additives in nasal formulations should not interfere with the self-cleaning capacity of the nose by the ciliary epithelium. Measured *in vitro* on human adenoid tissue with a photoelectric method, STDHF was found to induce ciliostasis at concentrations of 0.3% (w/v) and higher. STDHF (0.3%) is less ciliostatic than laureth-9 (0.3%) or deoxycholate (0.3%). Glyco- and taurocholate (0.3%) show only very mild effects on nasal ciliary movement. Human insulin (1%) has no ciliostatic potency *in vitro*, whereas a combination of human insulin (1%) and STDHF (1%) is ciliostatic but not as potent as STDHF (1%) alone.

KEY WORDS: Ciliotoxicity; sodium taurodihydrofusidate (STDHF); bile salts; laureth-9; insulin.

INTRODUCTION

Recent investigations have demonstrated that the nasal mucosa allows effective drug absorption (1). Particularly for peptide drugs, nasal delivery is a promising alternative to parenteral administration (1,2). However, because of their high molecular size and hydrophilic properties at physiological pH values, peptides show poor transport characteristics across the hydrophobic membrane barriers. The absorption efficiency of intranasally administered peptides can be improved by the use of absorption enhancers, such as bile salts, laureth-9, or fusidate derivatives (3–6). Sodium taurodihydrofusidate (STDHF) has recently reported to be a potent enhancer of intranasally administered insulin in sheep (5).

When considering nasal drug delivery, the effects of drug and additives on nasal functions should be tested at an early stage. Because the self-cleaning capacity of the nose by the ciliary epithelium is necessary to remove dust, allergens, and bacteria, it should not be influenced by nasal medication. Ciliary movement is a major factor for the mucociliary clearance in the upper airways (7). From patients with "immotile cilia syndrome" it is known that chronic nasal ciliary arrest leads to recurrent infections of the airways (9). Many drugs and additives inhibit nasal ciliary movement as demonstrated *in vitro*. For instance, lipophilic and mercuric preservatives, antihistamines, propranolol, and dihydroxy bile salts are ciliostatic agents, all reducing the ciliary beat frequency to 0 within 20 min (8). It is further important to investigate whether a ciliostatic effect is reversible after withdrawal of drug exposure. Nasal absorption enhancers should be devoid of any serious ciliotoxicity, and the feasibility of nasal drug administration may depend largely on the effects on the ciliated epithelial tissue. The objective of the present report is to study the *in vitro* effect on human nasal ciliary beat frequency (CBF) of the recently established absorption promotor STDHF in relation to bile salts and laureth-9. In addition, the effects of nasal insulin formulations with or without STDHF on nasal ciliary movement were investigated.

MATERIALS AND METHODS

Materials

Sodium tauro-24,25-dihydrofusidate (STDHF) and human insulin were supplied by California Biotechnology Inc. (Mountain View, CA). Deoxycholic acid (Lot 126F-0175), taurocholic acid (Lot 47F-5035), glycocholic acid (Lot 26F-5025), and laureth-9 (polidocanol; Lot 123F-0201) were from Sigma (St. Louis, MO). Locke–Ringer (LR) is a sterilized isotonic solution of the following composition per liter aqua: NaCl, 7.72 g; KCl, 0.42 g; $CaCl_2 \cdot 2H_2O$, 0.16 g; dextrose, 1.00 g; and $NaHCO_3$, 0.15 g. All substances were of pharmaceutical quality.

Nasal Ciliary Beat Frequency

Ciliary beat frequency (CBF) was measured on human adenoid tissue with a photoelectric registration device as described earlier (10). Briefly, a light beam is transmitted through the moving cilia, and after magnification by a microscope the flickering light is projected on a photocell. The

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electrical signal, generated by this photocell, is visualized by an oscilloscope and the frequency of this signal is estimated electronically and displayed. Stock solutions in distilled water were prepared of STDHF, deoxycholate, glycocholate, taurocholate, and laureth-9 (1.5%, w/v). Before CBF measurement the stock solutions were diluted five times with LR. These test solutions (0.3%, w/v) were adjusted to pH 7.4. Thereafter the tubes were vortexed for 1 min. Also, STDHF concentrations from 0.1 to 1% (w/v) were prepared by dissolving the appropriate amount of STDHF in LR. Formulations of insulin (1%, w/v) with or without STDHF (1%, w/v) were prepared in LR. Control experiments (blank) were performed in pure LR.

All experiments were done at 30°C. When the CBF reached zero, the ciliated tissue was washed in LR to investigate eventual reversibility of the CBF.

All results were recorded as percentages of the initial frequencies.

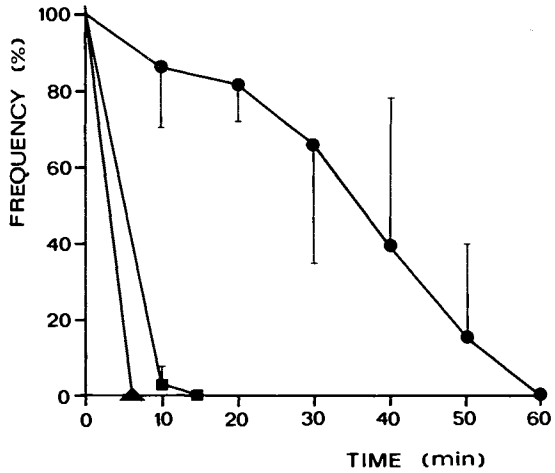

Fig. 1. Time versus frequency plot (mean ± SD) of cilia in solutions of STDHF, 0.3% (—●—); laureth-9, 0.3% (—▲—); and deoxycholate, 0.3% (—■—).

RESULTS AND DISCUSSION

The results of the *in vitro* nasal CBF measurements on human adenoid tissue are presented in Table I. From these data it is evident that the inhibition of the CBF by STDHF increases with increasing STDHF concentrations. STDHF at concentrations of 0.1 and 0.2% (w/v) shows minor effects. A breakpoint with respect to ciliotoxicity appears to be at a concentration of 0.3% (w/v). Higher concentrations (0.5 and 1%) cause ciliostasis, leading to complete CBF inhibition within 10 min; this ciliostasis is irreversible. Nevertheless, using similar concentrations of 0.3% (w/v), STDHF appears to be less ciliostatic than the well-known nasal absorption promoters deoxycholate and laureth-9, which both cause a very rapid irreversible ciliostasis (Fig. 1). The absorption enhancers glycocholate and taurocholate exhibit a very mild ciliostatic activity (Table I). The latter results obtained with human nasal ciliated tissue are in accordance with previously reported studies using chicken ciliated embryonal tracheal tissue (11) and confirm that dihydroxy bile salts (e.q., deoxycholate) are more ciliostatic than trihydroxy bile salts (e.q., glyco- and taurocholate). Our presented CBF data are also in agreement with the previously described effects of these enhancer compounds on rabbit erythrocyte lysis (5). STDHF in that assay is less lytic than laureth-9 or deoxycholate.

The observed critical concentration of STDHF 0.3% (w/v) may exceed the critical micelle concentration of STDHF, which is in the range of 0.2% in 0.01 *M* Na^+ (5). $T_{50\%}$, defined as the time at which the ciliary beat frequency drops to 50% of its initial value, for 0.3% (w/v) STDHF is about 40 min. For the bile salt cholate a linear relationship has been demonstrated between the $T_{50\%}$ and the cholate concentration (11). For STDHF, however, such a relationship was not established because of the abrupt ciliostasis seen at 0.5% (w/v). Human insulin (1%, w/v) does not have any effect on the nasal ciliary beat frequency. The combination of insulin (1%, w/v) with STDHF (1%, w/v) is ciliostatic although not as potent as STDHF 1% (w/v) alone (Fig. 2). These results indicate that the addition of insulin reduces the *in vitro* ciliostatic properties of STDHF. A possible explanation for this phenomenon may be that the polypeptide insulin in the

Table I. Effects of Various Absorption Enhancers and Insulin on Human Nasal Ciliary Beat Frequency (CBF)

	% (w/v)	Time (min)[a] 10	20	30	40	50	60	(*n*)
STDHF	0.1	93 ± 4	98 ± 5	94 ± 4	92 ± 6	90 ± 9	90 ± 9	(4)
STDHF	0.2	90 ± 2	92 ± 6	92 ± 9	88 ± 11	82 ± 11	82 ± 13	(4)
STDHF	0.3	86 ± 14	82 ± 8	64 ± 31	39 ± 39	16 ± 25	0	(8)
STDHF	0.5	0	0	0	0	0	0	(4)
STDHF	1.0	0	0	0	0	0	0	(4)
Laureth-9	0.3	0	0	0	0	0	0	(8)
Deoxycholate	0.3	2 ± 4	0	0	0	0	0	(8)
Taurocholate	0.3	96 ± 7	93 ± 6	90 ± 7	90 ± 10	90 ± 8	90 ± 8	(8)
Glycocholate	0.3	89 ± 7	87 ± 10	88 ± 8	87 ± 9	87 ± 10	85 ± 11	(8)
Human insulin	1.0	99 ± 3	99 ± 3	99 ± 4	99 ± 6	97 ± 10	93 ± 7	(4)
Human insulin with STDHF	1/1	17 ± 32	10 ± 28	3 ± 8	0	0	0	(8)

[a] All values are presented as percentages of the initial frequencies (t_0 = 100%) and are the mean ± SD for the number of experiments given in parentheses (*n*).

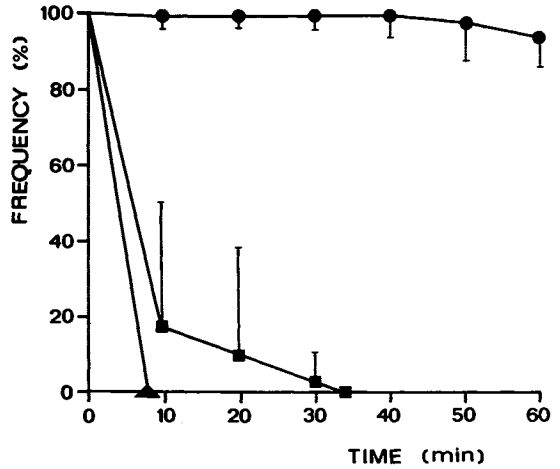

Fig. 2. Time versus frequency plot (mean ± SD) of cilia in solutions of human insulin, 1% (—●—); STDHF, 1% (—▲—); and human insulin with STDHF, 1/1% (—■—).

STDHF micelle (5) prevents the micelle from interacting with the nasal cilia because of steric hindrance or reduction of overall hydrophobicity.

In conclusion, with respect to the effects on nasal ciliary movement *in vitro*, we have shown that the potent absorption enhancer STDHF, although ciliostatic at concentrations of 0.3% (w/v) and higher, is less ciliostatic than laureth-9 or deoxycholate. On the other hand, at similar concentrations of 0.3% (w/v) STDHF reduces the nasal ciliary beat frequency to a greater extent than the trihydroxy bile salts glyco- and taurocholate. Use of this *in vitro* human adenoid tissue model allows for relative comparisons of the ciliostatic potential of various excipients and a study of concentration-dependent effects.

Evidence is available for a significant correlation between CBF and mucociliary clearance time, suggesting that CBF is the main factor in the nasal mucociliary clearance in human volunteers (7). The mucociliary clearance ranges between 3 and 20 min in healthy persons (7). The present knowledge of nasal physiology does not give a clear indication to what extent ciliostatic formulations are harmful *in vivo*. In contrast to our *in vitro* results, preliminary experiments by other investigators with clinical formulations containing STDHF at concentrations between 0.5 and 1.5% (w/v) show no change in mucociliary clearance times when assessed by the standard saccharine taste test methodology (12).

Thus the *in vivo* effects on nasal ciliary functioning of formulations containing STDHF seem less dramatic than the presented *in vitro* effects, because of the dilution of the formulation by the nasal mucus and elimination by the mucociliary clearance. The mucociliary clearance, however, will be largely inhibited when the ciliary movement is rapidly diminished in an irreversible way. Therefore we feel that long-term application of irreversibly ciliostatic formulations in nasal drug delivery should be avoided.

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REFERENCES

1. Y. W. Chien. *Transnasal Systemic Medications. Fundamentals, Developmental Concepts and Biomedical Assessments*, Elsevier Science, Amsterdam, 1985.
2. D. A. Eppstein and J. P. Longenecker. *CRC Crit. Rev. Ther. Drug Carrier Syst.* 5:99–139 (1988).
3. A. E. Pontiroli, M. Alberetto, A. Secchi, G. Dossi, I. Bosi, and G. Pozza. *Br. Med. J.* 284:303–306 (1982).
4. A. C. Moses, G. S. Gordon, M. C. Carey, and J. S. Flier. *Diabetes* 32:1040–1047 (1983).
5. J. P. Longenecker, A. C. Moses, J. S. Flier, R. D. Silver, M. C. Carey, and E. J. Dubovi. *J. Pharm. Sci.* 76:351–355 (1987).
6. M. J. M. Deurloo, W. A. J. J. Hermens, S. G. Romeijn, J. C. Verhoef, and F. W. H. M. Merkus. *Pharm. Res.* 6:853–856 (1989).
7. G. S. M. J. E. Duchateau, K. Graamans, J. Zuidema, and F. W. H. M. Merkus. *Laryngoscope* 95:854–859 (1985).
8. W. A. J. J. Hermens and F. W. H. M. Merkus. *Pharm. Res.* 4:445–449 (1987).
9. B. A. Afzelius. *Int. Rev. Exp. Pathol.* 19:1–43 (1979).
10. H. J. M. van de Donk, J. Zuidema, and F. W. H. M. Merkus. *Rhinology* 18:93–104 (1980).
11. G. S. M. J. E. Duchateau, J. Zuidema, and F. W. H. M. Merkus. *Int. J. Pharm.* 31:193–199 (1986).
12. J. P. Longenecker. Personal communication.