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The influence of insulin and some excipients used in nasal insulin preparations on mucociliary clearance

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Summary

The potential local toxicity to the nasal mucosa of insulin, various absorption promoters and vehicles has been tested in the frog palate model. The influence on mucociliary transport rate was used as an estimate of toxicity. Full inhibition of this rate was observed with 1% L-α-lysophosphatidylcholine, 1% polyoxyethylene-9-lauryl ether, 1% sodium deoxycholate and 1% sodium dihydrotaurofusidate, whereas 1% sodium glycocholate and 1% didecanoyl-L-α-phosphatidylcholine had no effect. 0.1% sodium dihydrotaurofusidate increased the mucociliary transport rate and the insulin formulation Novolin® reduced the rate slightly, but these latter effects were always reversible.

Introduction

Recent investigations have demonstrated that the nasal mucosa might represent a potential site for insulin absorption, as the surface of the mucosa is large and well provided with blood vessels (Chien et al., 1989). The treatment of diabetic patients requires that they receive daily insulin and therefore it is important to determine the effects of intranasal administration of insulin and any vehicles on nasal mucociliary clearance.

The epithelium of the upper respiratory tract is covered by many hair-like cilia beating in a co-ordinated manner within the periciliary fluid beneath a layer of viscoelastic mucus, the whole comprising the mucociliary apparatus. Mucociliary clearance is an important defence mechanism of the human body against inhaled dust, allergens and micro-organisms. Although ciliary beating is a major component of mucociliary clearance in the nose, the influence of mucus secretion, swallowing, sniffing and gravity must also be considered.

This self-cleaning capacity of the nasal cavity can either be reduced or increased by both drugs Hermens and Merkus, 1987) and additives (Ryde, 1961; Batts et al., 1989) in nasal preparations. Hermens et al. (1987) state that the mucociliary clearance should not be decreased by nasal medication. A decreased motility may be predictive of a local toxic influence on the epithelial mucosal cells. However, if the rate of clearance is slightly

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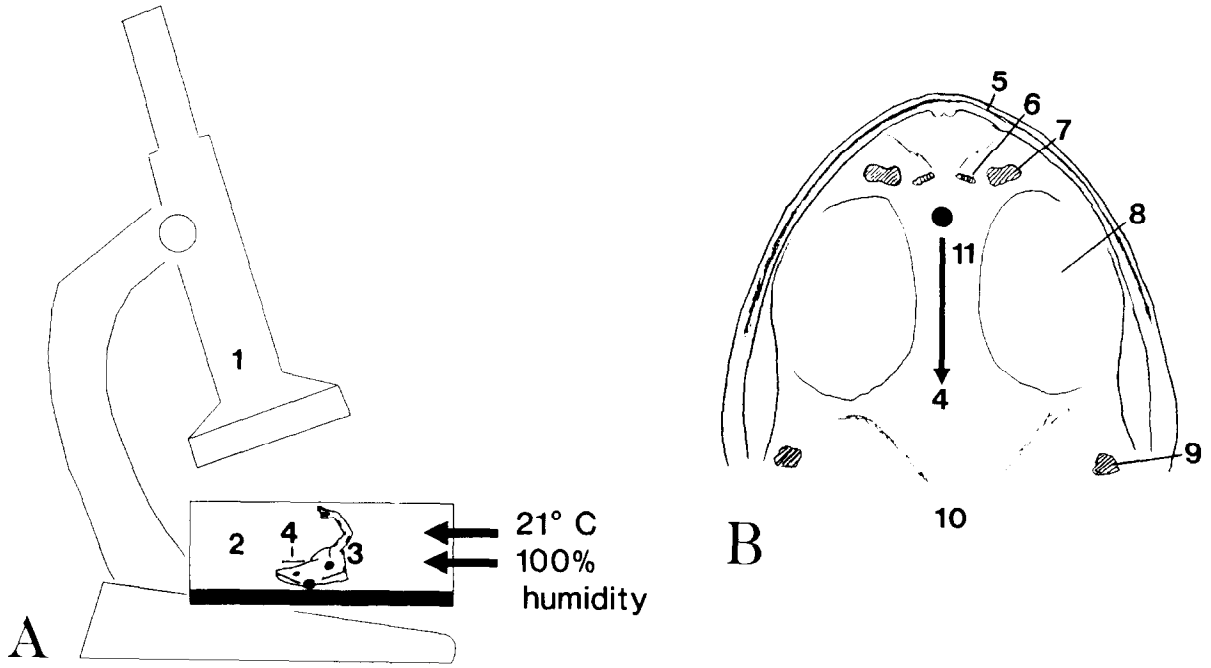

Fig. 1. (A) The experimental set-up for the frog palate model and (B) The roof of the frog's mouth: (1) Stereo microscope; (2) chamber, maintained at 21° C with a relative humidity of 100%; (3) pithed frog with upper palate exposed; (4) region of transport for the graphite particles; (5) maxillary teeth; (6) vomerine teeth; (7) nasal passage (choana); (8) protrusion of eyeballs; (9) eustachian tube leading to the middle ear; (10) entrance to oesophagus; (11) hard palate.

decreased or halted in a reversible manner, such retardation might be useful and could result in an increased contact time between the active drug and nasal mucosa, thereby increasing the bioavailability.

The aim of the present investigation was to study the influence of insulin and some of the vehicle systems and absorption promoters, which have been used for insulin, on the mucociliary clearance in the frog palate model.

Materials and Methods

Reagents and solutions

The control solution for all compounds was phosphate-buffered saline (PBS) prepared in distilled water. The test solutions were prepared by dissolving the required amount of compound in the appropriate control solution. Zinc-free (monomeric) human insulin (9.4 mg/ml) and Novolin®, with and without insulin, were kindly provided by Novo Nordisk (Bagsvaerd, Denmark). L-α-Lysophosphatidylcholine (LPC) and didecanoyl-L-α-phosphatidylcholine (DDPC), polyoxyethylene-9-lauryl ether (Laureth-9), sodium deoxycholate (SD) and sodium glycocholate (SG) are commercially available from Sigma (St. Louis, MO, U.S.A.) and sodium-dihydrotaurofusidate (SDHTF) was kindly provided by Leo Pharmaceuticals (U.K.). The absorption promoters were dissolved (except for DDPC, which was used as a suspension) at a concentration of 1 and 10 mg/ml.

Methods

Fig. 1 shows the experimental arrangement for the frog palate model. Transport rate was measured in vitro using a modification of the method described by Sade et al. (1970). The frog (*Rana pipiens*) was beheaded, the upper palate was exposed and introduced into a transparent chamber maintained at 21° C with a relative humidity of 100%. The palate surface was observed through a stereo-microscope (Wild M3C, Wild Leitz,

Glostrup, Denmark) fitted with a calibrated eyepiece.

Control values ($n = 6–10$) were obtained for each experiment by applying 0.2 ml of PBS to the palate and leaving it in contact for 2 min before draining off. The transport rate was then measured by recording the time taken for graphite particles to travel a given distance along the midline of the palate (usually 1–3 mm), from the anterior part of the hard palate, just behind the vomerine teeth, towards the posterior part, near the oesophagus. The test compound was applied in a similar manner.

To evaluate the influence of each test substance, each frog was used as its own control and the calculations were performed using paired data in combination with Student's *t*-test.

Results and Discussion

The results are given in Table 1 which shows that the mucociliary transport rates were measured up to 30 min after the application of PBS or test solutions, respectively. After the administration of PBS to the palate, the values of mean mucociliary transport rate, although variable, remained relatively constant for each palate within a narrow range of 0.20–0.30 mm/s. The application of some test solutions resulted in transport rates which were very different from the control data (PBS).

The application of human monomeric insulin showed no significant effect on the mucociliary transport rate. This finding shows that insulin does not influence the mucociliary transport rate. However, it must be remembered that results obtained with this model do not necessarily relate to the effect of insulin in humans, since it is known that frog and human insulin are different.

Immediately after the application of LPC, Laureth-9, SD and SDTF the mucociliary transport was irreversibly halted (100%). These absorption promoters are all being tested in intranasal preparations (Moses et al., 1983; Saltzman et al., 1985; Ilum et al., 1988) and their effect may be directly on the cilia, the mucus, the membrane or any combination of these. In some studies with LPC the mucus structure appeared to be reduced since it slid down the palate. These findings are in accordance with a study by Martin et al. (1978), where the effect of LPC was studied on human bronchial mucus. These workers found that the viscoelastic nature of the mucus was decreased, showing that structure breakdown had occurred. The results of the current investigation would

TABLE 1

The influence of insulin, some absorption promoters and an insulin formulation on the mucociliary transport rate, measured on a frog palate model as the average speed (± S.D.) of applied graphite palate particles towards the oesophagus (statistical test: Student's t-test with paired data)

Compound	*N*	Speed before (mm/s)	Speed after (mm/s)	Change (%)	Comments
0.9% insulin	8	0.30 ± 0.07	0.29 ± 0.12	−2	
1% L-α-lysophophatidylcholine	6	0.23 ± 0.12	0.00 ± 0.00	−100	irreversible change
1% L-α-phosphatidylcholine, didecanoate	9	0.26 ± 0.07	0.23 ± 0.03	−12	
Novolin® (with insulin)	6	0.26 ± 0.07	0.18 ± 0.11	−29	
Novolin® (without insulin)	6	0.29 ± 0.08	0.10 ± 0.08	−63	significant change [a]
1% polyoxyethylene-9-lauryl ether	6	0.22 ± 0.11	0.00 ± 0.00	−100	irreversible change
1% sodium deoxycholate	6	0.24 ± 0.05	0.00 ± 0.00	−100	irreversible change
0.1% sodium dihydrotaurofusidate	6	0.21 ± 0.08	0.30 ± 0.09	+47	significant change (reversible) [b]
1% sodium dihydrotaurofusidate	6	0.28 ± 0.06	0.00 ± 0.00	−100	irreversible change
1% sodium dihydrotaurofusidate	9	0.26 ± 0.07	0.25 ± 0.09	−2	

[a] Significance level: $P < 0.05$. Two applications resulted in irreversible 100% decrease in the mucociliary movement.
[b] Significance level: $P < 0.05$.

suggest that Laureth-9 and SDTF would be poorly tolerated by human subjects. Studies in healthy volunteers have shown that LPC, Laureth-9, SDC and SDTF cause local irritation among the subjects tested which was manifested by stinging, congestion and rhinorrhea (Moses et al., 1983; Saltzman et al., 1985). Such absorption promoters have also been shown to cause changes to microvilli (Hirai et al., 1978) and furthermore, also have been shown to destroy the ciliary membrane (Simmers and Gibbons, 1971).

When 0.1% SDTF was administered to the palate, the mucociliary transport rate was increased significantly and this effect was always reversible. This could be explained by the ability of SDTF to increase mucus secretion (Moses, 1987) by the palate and stimulate ciliary beat frequency. The efficiency of mucociliary clearance is dependent upon the quantity and viscoelastic properties of the periciliary fluid and mucus as well as the number, beat frequency and coordination of the cilia (Mygind et al., 1982). An increase in mucociliary clearance could be argued to be unwanted when insulin or other drugs are administered into the nasal cavity. The clearance half-time would be decreased and thereby the contact time for the drug on the mucosal epithelia would be shortened. The application of 0.1 and, especially, 1% SDTF induced a remarkable increase in the amount of mucus secreted by the palate.

SG did not cause any significant change in the mucociliary transport rate. Human studies on intranasal administration of insulin have shown that SG is less irritating than other bile salts. Volunteers have reported no side effects and expressed a preference for intranasal insulin with SG as an absorption promoter (Pontoroli et al., 1982), relative to subcutaneous injection.

Only one intranasal insulin preparation, which at present is being tested in clinical trials (Novolin®, from Novo Nordisk, Denmark), was tested in the present study, as well as its absorption promoter, DDPC (Hansen et al., 1988). Surprisingly, the formulation without insulin decreased the mucociliary transport rate more than the final formulation with insulin or the absorption promoter DDPC alone. DDPC was found to have no significant effect on the mucociliary transport rate. Novolin® with insulin was found to decrease the rate moderately, but not significantly, and the effect was always reversible. Novolin® without insulin, however, reduced clearance rate significantly and in two results, the mucociliary transport rate was halted irreversibly. The reversible reduction in the transport rate, after the administration of the Novolin® formulation with insulin, could be of benefit in the absorption of insulin through the nasal epithelia since this might result in a longer contact time of the peptide at the absorption site.

It is important that the drug and the absorption enhancer or the final formulation should not affect the physiological function of the nasal mucociliary system. Of the absorption promoters investigated, SG, DDPC and 0.1% STDF appeared the least toxic. Irreversible ciliotoxicity was observed with LPC, Laureth-9, SD and 1% STDF.

In the model used in this work, the cilia are protected by an intact layer of mucus. The results with the frog palate model seem to correlate with those obtained with the ciliary beat frequency model described by Hermens et al. (1990). Comparing results from human nasal irritation studies with the results reported here, the frog model seems to be able to identify the least irritating promoter and may provide a basis for the selection of a nontoxic enhancer.

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