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Effects of insulin and nasal absorption enhancers on ciliary activity

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Summary

The potential ciliotoxicities of insulin and of the protein absorption enhancers, aprotinin and sodium cholate, were investigated by monitoring the decay in beat frequency of rat cilia exposed to them. Comparison of the area under the ciliary beat frequency decay curves indicates that insulin is relatively non-ciliotoxic over the time exposures studied. Aprotinin, a peptidase inhibitor and sodium cholate, a surface active bile salt, were both highly ciliotoxic. When admixed those two absorption enhancers showed additive rather than synergistic ciliotoxicity. The data suggest that those compounds exert a direct toxic effect on the cilial membranes. Use of those additives in intranasal formulations is therefore contraindicated.

Introduction

The nasal route of drug administration is most often used for the delivery of locally acting drugs, such as nasal decongestants and anti-allergic compounds (Li Wan Po, 1990). More recently, this route has been explored as a possible portal for the delivery of peptide and protein drugs (Zhou and Li Wan Po, 1991). Indeed, a number of peptide and protein drugs are already available as intranasal formulations, including oxytocin, calcitonin and vasopressin analogues. Although the bioavailabilities of such compounds are generally low (Pontiroli et al., 1989; Hussain et al., 1990), some studies show that clinical response is satisfactory (Sjoberg and Luft, 1963; Overgaard et al., 1989).

To enhance bioavailability absorption enhancers and peptidase inhibitors (Hussain et al., 1990) are being investigated.

One area of concern is the potential damage which such formulations can cause to the nose (Su et al., 1984; Chien and Chang, 1987). Risk of damage is particularly enhanced in the presence of absorption enhancers which may exert their effects through disruption of cell membranes (Hirai et al., 1981; Hersey and Jackson, 1987). Indeed, dihydroxy bile salts which are commonly

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considered as potential absorption enhancers have been shown to be ciliotoxic (Duchateau et al., 1986) and to induce extensive loss of epithelia (Hersey and Jackson, 1987) in animal studies. In humans, intranasal bile salts cause a burning sensation (Gordon et al., 1985).

In this study we report on studies investigating the possible ciliotoxicity of insulin and two potential nasal absorption enhancers, aprotinin and sodium cholate.

Materials and Methods

Insulin (Actrapid 100 IU/ml) was purchased from Novo Industry (Copenhagen, Denmark) and sodium cholate from Sigma Chemical Co. (St. Louis, MO). Aprotinin (Trasyl®) 1000 KIU/ml (kallikrein inactivator units) was kindly donated by Bayer (Leverkusen, Germany).

The rat trachea were incubated in Locke-Ringer solution (LR) made up from NaCl, 7.72 g; KCl, 0.42 g; $CaCl_2 \cdot 2H_2O$, 0.16 g; $NaHCO_3$, 0.15 g; dextrose, 1.0 g; and distilled water to 1000 ml. The pH 7.4 solution was sterilised at 110°C for 30 min before use. All the test solutions were made up in LR solution.

Tracheal rings were prepared using tracheas removed from freshly killed adult male Wistar rats weighing 300–400 g. Each trachea was cut into rings about 0.5 mm thick with the ciliated epithelium still intact. The rings were maintained in 5 ml LR control solution for at least 30 min, at 37°C prior to use to stabilise ciliary beats.

The ciliated tracheal rings were examined using an inverted Olympus (CK2-TRP) binocular microscope equipped with a compact video camera (Hitachi KP-143) connected to a Hitachi video monitor. Ciliary movement was recorded on an Akai (model 425-EK) video cassette recorder (VCR) for later analysis. The preparations were examined using a × 20 lens and a × 10 eyepiece.

The signal produced by the beating cilia was picked up by a photo-sensitive probe from the video monitor and was converted by fast Fourier transform into a frequency spectrum, using an Opus PC III microcomputer with on-board A/D converter. In the present study, the ciliary beat frequency (CBF) was measured every 30 s for up to 2 h.

Each tracheal ring was observed at three different sites. Hence, mean values of CBF were from nine different areas in three individual rats. After measurement of the control ciliary beat frequency, the control solution (LR) was replaced by LR solution containing the various drugs and/or absorption enhancers. In between test solutions, the chamber was rinsed several times with LR control solution.

Human insulin was used as a model protein drug and aprotinin and sodium cholate as possible nasal absorption enhancers.

Statistics for quantifying ciliotoxicity

When the cilia are exposed to toxic substances, the ciliary beat frequency (CBF) slows down relative to the initial values. Therefore, when the CBF is continuously monitored, damage to the cilia can be seen as a CBF-time decay curve. The more toxic the compound, the sharper is the decline in CBF with time (Van de Donk et al., 1982). Early studies used CBF measurements at specific time points for comparative purposes such as when comparing the toxicities of various compounds. In more recent literature, CBF-time curves are displayed for such purposes (Tamaoki et al., 1989). In a recent report, we advocated the wider use of area under the curve when comparing drug responses over time (Chan and Li Wan Po, 1992), in line with the growing recognition that summary statistics should be used when appropriate (Matthews et al., 1990). In this study, the area under the curve is defined as the area between the CBF = 100% and the CBF-time curve. We refer to this area as the complement of the area under the curve (CAUC), since in most other kinetic studies the area under the curve is the area between the abscissa and the relevant curve. The area under the curve was calculated using the trapezoidal rule and a Lotus 1-2-3® Macro (Chan and Li Wan Po, 1992).

Factorial study

The joint effects of aprotinin, a peptidase inhibitor and sodium cholate (a hyper-permeability-inducing agent) were studied using a 2^2 facto-

rial design similar to that previously described (Gilliland et al., 1992) and detailed in standard texts (Davies, 1956). The two concentrations of sodium cholate were 5.69 and 11.37 mM while those of aprotinin were 1000 and 2000 KIU.

All statistical analyses were performed using the generalized linear model and the computer packages Minitab® and SAS®.

Results and Discussion

Insulin was relatively mild on the cilia. An exposure of the cilia to 1% of the 100 IU/ml solution of insulin for 2 h produced only small decreases in ciliary beat frequency. Indeed, an initial minor decrease in CBF in the first 15 min is followed by recovery to the initial values over the next 2 h. We therefore used that basic insulin formulation to examine the effect of addition of aprotinin and sodium cholate on the CBF.

The curves in Fig. 1 show that interestingly the CBF was relatively unaffected by the aprotinin and sodium cholate combinations for about 25 min. There was then an abrupt decay in CBF with the cilia exposed to the high concentrations of aprotinin and sodium cholate. The other combinations produced similar profiles but after longer exposures. The basis for these unusual profiles is unknown but the kinetics suggest that the additives probably exert their toxicity by a direct irritant effect rather than by a receptor-based mechanism.

Analysis of variance appropriate for a 2^2 factorial design showed that while both sodium cholate and aprotinin produced a dose-dependent decrease in CBF, there was no interaction between those absorption enhancers. In other words, the increased effect produced by increasing the concentrations of one of the two compounds was not affected by the concentration of the other (Table 1). This suggests that both drugs probably exert non-specific ciliotoxicity in agreement with the lag phase data discussed above.

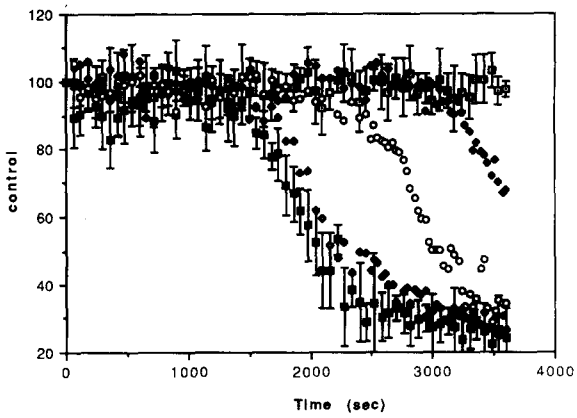

Fig. 1. Ciliotoxicity of sodium cholate and aprotinin combinations in the presence of 1% insulin (⊡) Control; (♦) 5.69 mM sodium cholate + 1000 KIU aprotinin; (○) 5.69 mM sodium cholate + 2000 KIU aprotinin; (◇) 11.32 mM sodium cholate + 1000 KIU aprotinin; (■) 11.32 mM sodium cholate + 2000 KIU aprotinin.

Conclusion

Protein and peptide drugs are poorly bioavailable by non-parenteral routes. The search for an effective oral delivery system for insulin has not abated since its discovery by Banting and Best

TABLE 1

Analysis of variance table for the effect of sodium cholate and aprotinin combinations on beat frequency of cilia exposed to a 1% insulin solution

Source	Sum of squares	Degrees of freedom	Mean of squares	*F* value	*P* value
Sodium cholate	42432909312	1	42432909312	199.44	< 0.001
Aprotinin	7068633600	1	7068633600	33.22	< 0.001
Interaction	130283200	1	130283200	0.61	0.44
Error	6808330240	32	212760320		
Total	56440336384	35			

(1922). Even for peptide drugs which are used effectively in practice, bioavailability is often poor. While the nasal route is an attractive alternative to the oral route for the delivery of such drugs, their ciliotoxicity and that of their potential absorption enhancers represent formidable outstanding challenges for formulation scientists. Until safer alternative absorption enhancers become available, effective nasal formulations for the systemic delivery of peptide and protein drugs will remain elusive.

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