Effects of Absorption Enhancers on Rat Nasal Epithelium in Vivo: Release of Marker Compounds in the Nasal Cavity

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Purpose. The assessment of the effects of nasal absorption enhancers on the rat nasal epithelium and membrane permeability *in vivo* after a single nasal dose of the enhancers. ***Methods.*** The release of marker compounds (protein, cholesterol and acid phosphatase) from the nasal epithelium was measured using a lavage technique. The nasal membrane permeability was determined after intravenous administration of a systemic tracer (FITC-albumin). ***Results.*** The effects of the absorption enhancers could be classified into four categories. The first consisted of HPβCD (5%), DMβCD (2%) and RAMEB (2%) and was not different from the control (physiological saline). For the second category, DMβCD (5%), effects were significantly higher than for the control. The third category, SGC (1%), was more active than DMβCD (5%) but less active than the last group. The fourth, most membrane damaging, category consisted of STDHF (1%), laureth-9 (1%) and LPC (1%). Administration of these three enhancers also resulted in release of acid phosphatase, indicating that severe membrane damage occurred. The release of cholesterol from nasal epithelium was largely dependent on the cholesterol solubilisation of the absorption enhancers. The amount of cholesterol released by laureth-9 and LPC was the largest. ***Conclusions.*** The results of this *in vivo* study are in agreement (i.e. similarity in rank order) with morphological and ciliotoxicity studies of nasal absorption enhancers, demonstrating that this *in vivo* model is a valuable tool to classify nasal absorption enhancers according to their effects on the rat nasal epithelium.

KEY WORDS: nasal absorption enhancers; cyclodextrins; bile salts; L-α-lysophosphatidylcholine; laureth-9.

INTRODUCTION

Absorption enhancers play an essential role in nasal drug delivery. In particular for peptides and proteins the use of absorption enhancers is often mandatory to reach an effective absorption from the nasal mucosa. Unfortunately, little is known about potential side effects of these substances, which is a drawback for the clinical application of absorption enhancers in nasal drug formulations. Looking more closely at the effects and the mechanisms underlying absorption enhancement of absorption enhancers on the nasal mucosa provides more information on their efficacy and safety (1). The mechanisms that lead to increased nasal drug absorption under the influence of enhancers are quite diverse and only partly understood. In some cases the solubility or the stability of the drug is increased, but absorption enhancers can also interact with the mucus layer, changing the mucus properties. Furthermore, the permeability of the nasal epithelium might also be increased due to interaction with the epithelial membranes.

Histological studies with rats have demonstrated the damaging effects of some absorption enhancers on the nasal epithelium *in vivo* and *in vitro* (2,3,4). Other methods study the effect of absorption enhancers on the mucociliary clearance. For this purpose an in vitro method has been developed that determines the influence of enhancers on the ciliary beat frequency of cilia of new-born chicken trachea and human adenoid tissue (5–6).

An *in situ* rat model measures the release of proteins, phospholipids and enzymes from rat nasal epithelium induced by cyclodextrins (7–8), bile salts (9) and strongly acidic pH values (10). It is based on the *in situ* perfusion model by Hirai et al. (11), and involves the perfusion of a solution of the absorption enhancer through the nasal cavity of a rat *in situ* for 1.5–6 h. This model provides a considerable amount of information on the effects of substances on the nasal epithelium, but its conditions are rather extreme compared to the circumstances in which nasal drug delivery usually takes place. The perfusion of a large volume of fluid through the nasal cavity via the oesophagus is completely different from the installation of 20 μl of fluid per nostril *in vivo*. A 1.5–6 h perfusion of a solution is long, considering that the nasal mucociliary clearance half-time in rats is only 5 min (12).

A new *in vivo* model is described in this paper to study and compare the acute effects of commonly used enhancers after a single nasal dose. The formulation was administered to each nostril and after a period of 15 min the nasal cavity was washed out by a perfusion via an oesophageal cannula. The effect of the absorption enhancer on the nasal epithelium was determined from the release of several marker compounds in the lavage. Additionally, the increase of the permeability of the nasal epithelium, as a result of the administration of an absorption enhancer, was measured by the leakage of a systemic tracer from the capillaries into the nasal cavity. The systemic tracer, fluorescence labelled albumin (FITC-albumin), was administered intravenously to the rat 5 min prior to the nasal dosage. Albumin has already been used to investigate increased rat epithelial permeability for macromolecules under the influence of neurological mediators, such as capsaicin or substance P (13). The aim of this study was to evaluate the possible harmful side effects of nasal absorption enhancers on the nasal epithelium and to classify the enhancers, according to the observed effects using this *in vivo* rat model.

MATERIALS AND METHODS

Chemicals

Hydroxypropyl-β-cyclodextrin (HPβCD, molar substitution 0.45) and Hypnorm, containing 10 mg fluanison and

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0.315 mg fentanyl citrate per ml 0.9% NaCl, were acquired from Janssen Pharmaceutica (Tilburg, The Netherlands). Pentobarbitone sodium (Nembutal) was purchased from Sanofi B.V. (Maassluis, The Netherlands) and dimethyl-β-cyclodextrin (DMβCD, degree of substitution 2.0) from Avebe (Foxhol, The Netherlands). Sodium taurodihydrofusidate (STDHF) was supplied by California Biotechnology Inc. (Mountain View, CA, USA) and randomly methylated β-cyclodextrin (RAMEB, degree of substitution 1.8) by Cyclolab (Budapest, Hungary). Fluorescein isothiocyanate (FITC) bovine albumin, containing 11.2 mol FITC per mol albumin was from Sigma (St. Louis, MO, USA). Sodium glycocholate (SGC), laureth-9 (L-9) and L-α-lysophosphatidylcholine (LPC) were also obtained from Sigma. All other reagents were of analytical grade.

Experimental

Male Wistar rats, weighing 210–240 g, were used. Rats were anesthetized with Hypnorm (0.8 ml/kg) subcutaneously and with Nembutal (0.33 ml/kg) intraperitoneally. The vena femoralis was cannulated to administer FITC-albumin. A 12 cm silicone cannula was inserted into the trachea to enable breathing. A second cannula of 20 cm silicone was inserted into the oesophagus into the posterior end of the nasal cavity and the nasal cavity was perfused through this cannula. The naso-palatine duct was sealed with cyaonoacrylate adhesive to prevent leaking of solution into the oral cavity. At the start of the experiment a bolus dose of 200 μl 10% (w/v) FITC-albumin solution in physiological saline was injected into the vena femoralis. Five min after this injection 20 μl of the enhancer solution per nostril was administered. Enhancer solutions were prepared in physiological saline. As control, 20 μl of saline per nostril was administered. After a 15 min incubation period the nasal cavity was perfused with physiological saline to wash out the biochemical markers released from the nasal epithelial membrane. The pump-rate was 2 ml/min and a total volume of 10 ml was perfused. The perfusate was collected in 5 fractions of 2 ml and stored at −4°C until analysis.

Enhancer Solutions

All enhancer solutions were prepared in physiological saline. The saline solution, containing 0.9% NaCl, was prepared with Millepore water. The following enhancers were studied: HPβCD (5%, w/v), DMβCD (2% and 5%, w/v), RAMEB (2%, w/v), SGC (1%, w/v), STDHF (1%, w/v), LPC (1%, w/v) and laureth-9 (1%, w/v). These concentrations were based on those frequently used in nasal drug delivery studies in animals and man.

Markers: Analytical Procedures

Protein Content. The protein content of the nasal lavage fluid was determined by the method of Lowry et al. (14), using bovine serum albumin as a standard.

FITC-Albumin. The FITC-albumin content of the samples was determined with fluorescence detection (excitation wavelength 498 nm, emission wavelength 520 nm). Separation of intact FITC-albumin from dissociated FITC was performed with size exclusion chromatography, using a 300 mm polymer HEMA-Bio size-exclusion column (Tessek, Prague, Tchechia). The mobile phase was 20 mMol NaH_2PO_4 in distilled water, adjusted to pH 10 with 1 N NaOH. The flow-rate was 1 ml/min.

Cholesterol. The amount of cholesterol in the nasal lavage fluid was determined with reversed phase HPLC, using a Chromsphere C18 column with a particle size of 5 μ, a length of 100 mm and an internal diameter of 3 mm (Chrompack, Bergen op Zoom, The Netherlands) and as eluent isopropanol:acetonitrile = 30:70 (v/v) at a flow-rate of 0.5 ml/min. Cholesterol was detected by UV absorption at 210 nm.

Acid Phosphatase Activity. The enzyme activity of acid phosphatase was measured with an enzyme kit (Easy test, Merck, Darmstad, Germany). This assay was based on measuring the conversion of p-nitrophenyl phosphate into p-nitrophenol. The detection limit of 2 ml samples was about 0.01 U/fraction. The amount of acid phosphatase activity of fractions 2–5 was below the detection limit. Therefore only the first fraction was used to determine the total amount of enzyme activity.

Cholesterol Solubility Experiments

Cyclodextrins increase the solubility of cholesterol by the formation of inclusion complexes. The increase in solubility of cholesterol is dependent on the degree and kind of substitution of the cyclodextrin. The solubility of cholesterol in the presence of HPβCD (3, 5, 7% w/v), DMβCD (1, 2, 3% w/v), RAMEB (1, 2, 3% w/v), SGC (0.5, 1, 1.5% w/v), STDHF (0.1, 1, 2.5% w/v), LPC (1% w/v) and laureth-9 (0.1, 1, 5% w/v) was determined. An excess of 20 mg cholesterol was added to a 1 ml enhancer solution and shaken for 15 days in an IKA-Vibrax VXR (Janke & Kunkel GMBH & Co. KG, Staufen, Germany) at ambient temperature. The solutions were centrifuged at 14,000 rpm for 30 min to remove undissolved cholesterol. The supernatant was again centrifuged and the cholesterol concentration of the resulting solution was determined. The amount of cholesterol dissolved by the enhancer concentrations was intrapolated from these data.

Statistics

The data of the different enhancers and the control (physiological saline) were compared by ANOVA testing. Before ANOVA testing was performed, it was verified that the requirements for ANOVA testing, i.e., normality of distribution and equality of variances, were met. The data were tested for normal distribution with a Kolmogorov-Smirnov test. For each marker the data were found to be normally distributed ($P > 0.05$). Then a Cochran test was performed to see whether the variances of the different groups of absorption enhancers were equal. For cholesterol and acid phosphatase the test showed equal variances for the treated groups with detectable marker levels. For protein and FITC-albumin the variances of the different groups were found not to be equal. Therefore, the data of these two markers were transformed to their square root. The transformed data appeared to be normally distributed according to Kolmogorov-Smirnov testing ($P > 0.05$), and these data were also found to have equal variances for both protein as well as FITC-albumin. For all further testing of protein and FITC-albumin

Table I. Amount of Marker Compounds Released After Administration of Absorption Enhancers

Enhancer	Protein (mg)	FITC-albumin (pMol)	Cholesterol (nMol)	Acid phosphatase (U)
Control	0.23 ± 0.17	6 ± 5	—*	—
HPβCD (5%)	0.37 ± 0.37	10 ± 1	6.83 ± 4.36	—
DMβCD (2%)	0.35 ± 0.13	17 ± 15	15.34 ± 8.42	—
RAMEB (2%)	0.26 ± 0.22	29 ± 34	18.20 ± 17.3	—
DMβCD (5%)	0.81 ± 0.30	45 ± 17	22.46 ± 8.94	—
SGC (1%)	1.16 ± 0.30	91 ± 34	10.11 ± 3.53	—
STDHF (1%)	1.64 ± 0.43	103 ± 45	30.16 ± 9.12	0.054 ± 0.033
LPC (1%)	2.12 ± 0.82	131 ± 61	50.50 ± 12.53	0.022 ± 0.016
Laureth-9 (1%)	2.43 ± 0.57	161 ± 79	44.34 ± 9.85	0.045 ± 0.022

Data are presented as the mean ± S.D. of 6–7 experiments.
* Nondetectable.

data the square root transformation was used. After verification that the requirements for ANOVA testing were met, the ANOVA test was performed. For all marker compounds, except acid phosphatase, the null-hypothesis (i.e. all absorption enhancers came from the same group) could be rejected. To further compare the effects of different absorption enhancers, multiple comparisons were performed for protein, FITC-albumin and cholesterol, using a Least Difference (LSD) test, with $P < 0.05$ as significance level. For all statistical calculations the computer program Statgraphics 2.1 was used.

RESULTS

Release of Protein

The protein release observed after nasal installation of the saline control was rather large, probably due from the washout of the mucus layer of the epithelium (Table I and Fig. 1). Administration of the absorption enhancers to the nasal cavity resulted in a significant increase in protein release for DMβCD (5%), SGC, laureth-9, STDHF and LPC (ANOVA: $P < 0.001$). Multiple comparison suggested the following homogenous groups: 1. physiological saline, HPβCD (5%), RAMEB (2%) and DMβCD (2%); 2. DMβCD (5%) and SGC (1%); 3. SGC (1%) and STDHF (1%); 4. STDHF (1%) and LPC (1%); 5. LPC (1%) and laureth-9 (1%). This grouping is graphically presented in Fig. 2.

Release of FITC-Albumin

The control (physiological saline) showed some leakage of FITC-albumin into the nasal cavity, indicating a spontaneous leaking of plasma proteins into the nasal cavity. The administration of enhancers increased the leaking of FITC-albumin into the nasal fluid (Table I and Fig. 3; ANOVA: $P < 0.001$). Multiple comparison with a least significance test gave the following homogenous groups for the FITC-albumin release (Fig. 2): 1. physiological saline, HPβCD (5%), DMβCD (2%) and RAMEB (2%); 2. RAMEB (2%) and DMβCD (5%); 3. SGC (1%), STDHF (1%) and LPC (1%); 4. STDHF (1%), LPC (1%) and laureth-9 (1%).

Release of Cholesterol

The release of cholesterol in the nasal fluid was not detectable for the control (Table I). The data for cholesterol release by the enhancer were not transformed because they met the requirements for ANOVA testing (Fig. 4; ANOVA: $P < 0.001$). Using a least significant difference test the following homogenous groups could be discerned: 1. HPβCD (5%), SGC (1%), DMβCD (2%) and RAMEB (2%); 2. DMβCD (2%), RAMEB (2%) and DMβCD (5%); 3. DMβCD (5%) and STDHF (1%); 4. laureth-9 (1%) and LPC (1%). This grouping is graphically presented in Fig. 5.

For the group of cyclodextrins, DMβCD and RAMEB increased the solubility of cholesterol more than HPβCD (Table II). *In vivo* DMβCD and RAMEB also released more

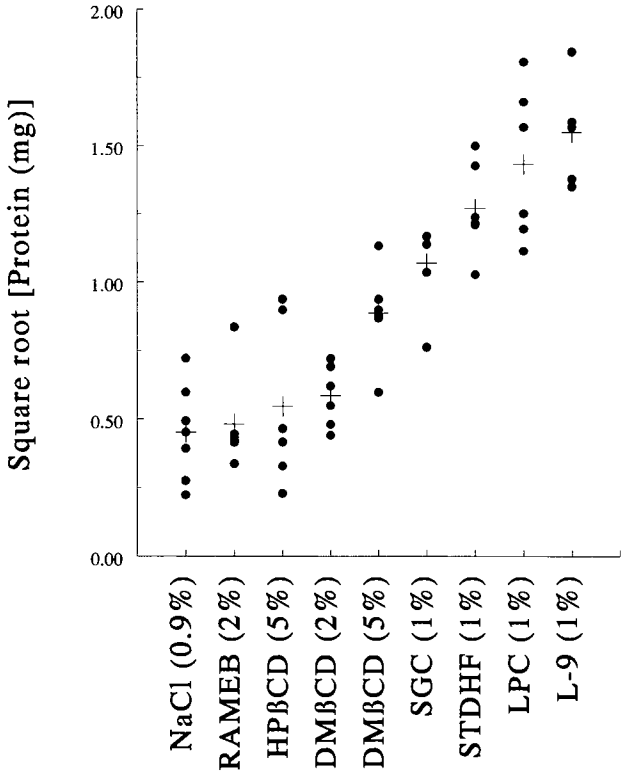

Fig. 1. Amount of protein released in rat nasal cavity after administration of absorption enhancers. The square roots of the individual data (●) and the mean (+) of the data (n = 6–7) are presented.

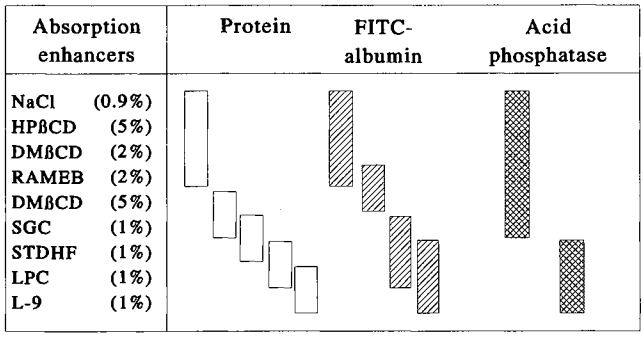

Fig. 2. Homogenous groups (LSD, $P < 0.05$) of absorption enhancers for the protein, FITC-albumin and acid phosphatase release from the rat nasal epithelium.

cholesterol from the nasal membrane than HPβCD. Laureth-9 and LPC were the most active in increasing the cholesterol solubility and these two enhancers also released significantly more cholesterol from the nasal membrane than other enhancers. SGC and STDHF were the least potent with respect to increasing the solubility of cholesterol. For the *in vivo* release of cholesterol SGC was part of the least effective group, which also included HPβCD, DMβCD (2%) and RAMEB (Fig. 4). The release of cholesterol from nasal membranes by STDHF, however, was significantly higher and it could be placed in the same group as DMβCD (5%), even though it was much less effective at solubilising cholesterol *in vitro* (Table II vs. Fig. 4).

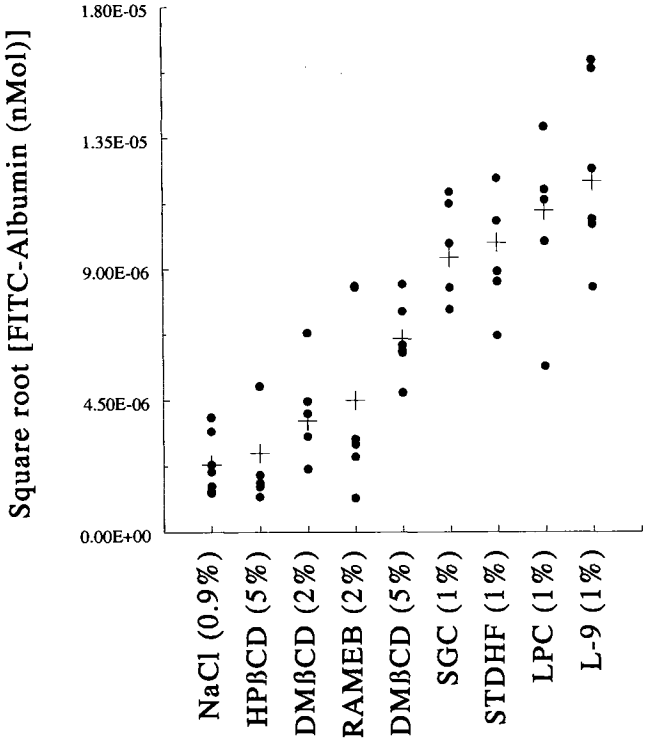

Fig. 3. Amount of FITC-albumin released in rat nasal cavity after administration of absorption enhancers. The square roots of the individual data (●) and the mean (+) of the data (n = 6–7) are presented.

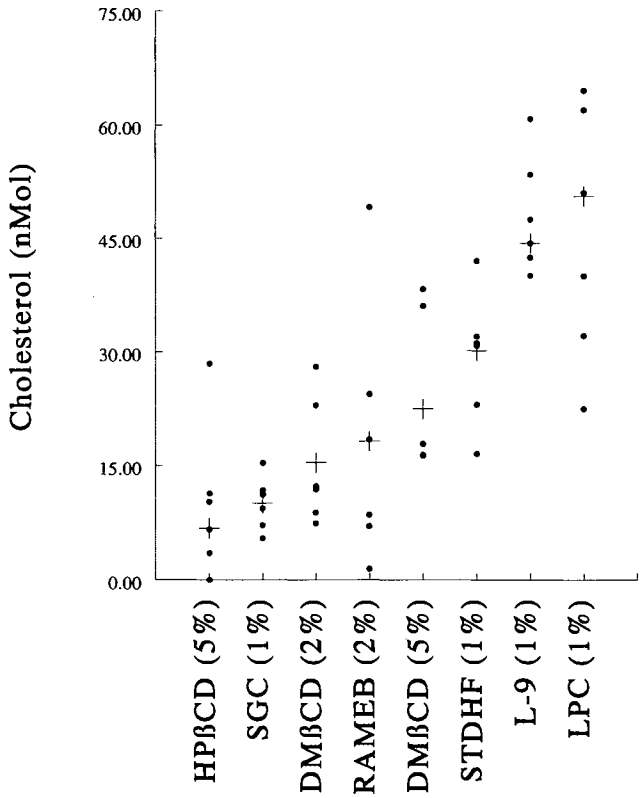

Fig. 4. Amount of cholesterol released in rat nasal cavity after administration of absorption enhancers. Individual data (●) and the mean (+) of the data (n = 6–7) are presented.

Release of Acid Phosphatase

The presence of the intracellular enzyme acid phosphatase in the nasal lavage is an indication of cell damage. For the control rats the release of this enzyme was not detectable. The amount of acid phosphatase in nasal lavage fluids of rats treated with cyclodextrins was also below the detection limit. Acid phosphatase appeared to be only detectable after administration of STDHF (1%), laureth-9 (1%) and LPC (1%) (Table I). ANOVA with $P < 0.05$ as significance level indicated that the null-hypothesis, i.e. the three

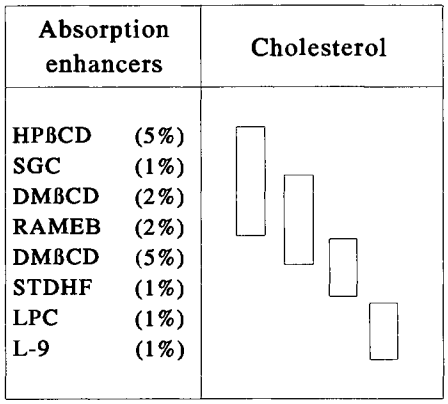

Fig. 5. Homogenous groups (LSD, $P = 0.05$) of absorption enhancers for the cholesterol release from the rat nasal epithelium.

Table II. Effect of Absorption Enhancers on Cholesterol Solubility

Absorption enhancer	Cholesterol concentration (mMol/l)
SGC (1%)	0.04
STDHF (1%)	0.11
HPβCD (5%)	0.78
DMβCD (2%)	1.70
RAMEB (2%)	2.17
LPC (1%)	3.34
Laureth-9 (1%)	30.20

Data are presented as the mean of 2 experiments.

enhancers came from the same group, could not be rejected (Fig. 2).

DISCUSSION

The observed effects of the absorption enhancers on the release of markers from the nasal epithelium in vivo can be used to elucidate the mechanisms behind their modus operandi. The protein detected in the nasal lavages may come from several sources: glycoproteins of the mucus layer, membrane proteins, enzymes, and plasma proteins. Histological studies have shown that application of LPC, laureth-9 and STDHF to the nasal epithelium of the rat results in a discharge of large amounts of mucus into the nasal cavity (2,3,4). They also showed that application of HPβCD resulted only in a slight increase of mucus on the surface of the epithelium (4). The observed large increase in protein release caused by LPC, laureth-9 and STDHF can therefore partly be attributed to the release of mucus glycoproteins from secretory cells.

Another source of proteins can be formed by plasma proteins. In the present study the effects of absorption enhancers on the exudation of plasma proteins were measured with FITC-albumin. The amount of plasma proteins leaking through the vascular endothelium and nasal epithelium increased after the application of absorption enhancers as is evident by the release of FITC-albumin. The spontaneous leaking of albumin in the controls and the observation that there were no intracellular enzymes detectable in the controls or in cyclodextrin treated rats both indicate that the release of FITC-albumin is not the result of extensive damage to the nasal epithelium. The leaking of large proteins through the fenestrae of the nasal capillaries and the pores of the endothelium was demonstrated by Watanabe et al. (15). The amount of FITC-albumin found in the nasal lavages was linearly correlated to the amount of protein. The correlation coefficient was 0.89, indicating that epithelial permeability and protein release are strongly related, but the amounts of FITC-albumin measured in the nasal lavage fluid presented less than 1% of the total amount of protein in the fractions. An alternative source of proteins are nasal membrane proteins which can be released when membrane extraction by absorption enhancers occurs. The amount of membrane protein in the fractions was not measured directly, but the release of cholesterol from membranes by absorption enhancers will most likely be accompanied by the release of other membrane components, for instance membrane bound proteins. The amount of membrane proteins released in the nasal fluid is presumably the largest for STDHF, LPC and laureth-9, because for these three enhancers severe membrane disruption was demonstrated by the release of the intracellular enzyme acid phosphatase.

In the controls and in the lavage fluid of rats treated with cyclodextrins no acid phosphatase activity could be detected. Histological studies disclosed that acid phosphatase activity is present in squamous and respiratory epithelium and is highest in the sensory cells of the olfactory epithelium, whereas mucus and cilia do not contain acid phosphatase activity (16). The release of acid phosphatase is therefore a primary indicator of olfactory epithelial damage. The release of cholesterol in the nasal fluid is most likely caused by the interaction of absorption enhancers with the nasal epithelium. The ability of DMβCD to interact with cholesterol in membranes has been reported (17), showing that DMβCD released larger amounts of cholesterol than HPβCD. This was also the case in the present study. These results are in accordance with our observation that less cholesterol was dissolved in the presence of HPβCD (5%) than in the presence of DMβCD (2%). STDHF caused a significantly higher release of cholesterol than HPβCD (5%) and DMβCD (2%), even though the solubility of cholesterol in the presence of STDHF was substantially smaller than in the presence of either cyclodextrin. From this observation it can be concluded that the interaction of STDHF with epithelial membranes is not based on the formation of inclusion complexes with cholesterol. The highest cholesterol release was caused by laureth-9 and LPC, indicating that these enhancers also have the most damaging interaction with nasal epithelial membranes.

The results from the release of protein, FITC-albumin, and acid phosphatase indicate that the different absorption enhancers can be classified into different categories according to their effects (Fig. 2). The first category includes enhancers causing effects comparable to the control, namely HPβCD (5%), RAMEB (2%) and DMβCD (2%). For the cholesterol release the effects of DMβCD (2%) and RAMEB (2%) were significantly different from the control (Fig. 5). A second category based on protein and FITC-albumin release is formed by DMβCD (5%). With respect to the results for acid phosphatase DMβCD (5%) was found to be comparable to physiological saline, showing that DMβCD is not destructive for the epithelial membrane. SGC (1%) forms the third category, it is comparable to the milder groups of enhancers with respect to acid phosphatase and cholesterol release, and to the more severe groups for protein and FITC-albumin release. Administration of SGC (1%) did not result in acid phosphatase release, placing SGC in the group that consists of the control and the cyclodextrins. For cholesterol SGC is also part of the group comparable to the control (Fig. 5), probably due to the rather limited capacity of SGC to increase cholesterol solubility in comparison to cyclodextrins (Table II). For protein and FITC-albumin release, however, SGC (1%) is comparable with STDHF (1%), an absorption enhancer belonging to the last category. The second and third categories of DMβCD (5%) and SGC (1%) can be joined into a so-called intermediate category.

The most membrane damaging category is composed of enhancers that display the most pronounced effects for all

markers except cholesterol, i.e. STDHF (1%), LPC (1%) and laureth-9 (1%). For the release of cholesterol the effects of STDHF (1%) are less severe than those of LPC (1%) and laureth-9 because STDHF is not an effective solubiliser of cholesterol.

By comparing the results of the present *in vivo* study with other studies it is apparent that the absorption enhancers displaying the smallest effects on the rat nasal epithelium, HPβCD (5%), RAMEB and DMβCD (2%), are also the least damaging in ciliotoxicity studies. HPβCD showed little or no damage to the nasal epithelial membrane in morphological studies (3) and also no substantial ciliostatic potency (18). For DMβCD (2%) the ciliary beat frequency decreased to 50% of its initial value after 50 min, and at this concentration DMβCD was already effective in increasing the intranasal absorption of insulin in rats (6).

For the enhancers of the intermediate category, SGC (1%) and DMβCD (5%), morphology and ciliotoxicity investigations also indicate that they cause less severe effects. Morphological studies disclosed that SGC (1%) had minor effects on epithelium morphology (19) and was less damaging than STDHF (1%) (2). Ciliotoxicity studies of SGC (1%) showed that at a 1% concentration the ciliary beat frequency decreased to 50% of its initial value after 50 min (1), while at a 5% DMβCD concentration reversible ciliostasis occurred after 30 min (6).

The enhancers of the most damaging category, STDHF, LPC and laureth-9, also exhibited the most damaging effects on the nasal epithelial membrane. In the present study, the release of intracellular acid phosphatase into the nasal fluid was demonstrated for STDHF, LPC and laureth-9, which indicates that severe cell damage occurred. In morphological studies of these enhancers epithelium disruption, cell loss and mucus discharge were observed (2,3,4). With scanning electron microscopy changes in ciliary morphology were noted for laureth-9 and LPC (2). STDHF resulted in less severe cell loss and epithelial perturbation than LPC (3). Previous ciliotoxicity studies of these enhancers demonstrated severe effects for laureth-9 (0.3%), LPC (0.5%) and STDHF (1%), i.e. complete and irreversible ciliostasis within 5 min (1,5). In clinical studies with human volunteers the local tolerability of STDHF (0.8%) was poor, giving rise to stinging sensations and lacrimation, and it was concluded that clinical application of STDHF as a nasal absorption enhancer was undesirable (20).

Another bile salt, sodium deoxycholate, was also tested in the present *in vivo* model (results not shown) and based on the release of marker compounds from the nasal cavity, it appeared to be belonging to the category of enhancers with the most membrane damaging effects. Morphological and ciliotoxicity studies also indicated that the effects of sodium deoxycholate on the nasal epithelium were very damaging (1,2).

In conclusion, the results of the present study show that this *in vivo* method in rats makes it possible to discriminate between different absorption enhancers with respect to their safety. Moreover, they also provide information on mechanistical aspects of nasal absorption enhancement. The following rank order can be established, with increasing toxicity: cyclodextrins < SGC < STDHF < LPC = laureth-9 = deoxycholate. This rank order correlates well with rank orders observed in morphological as well as ciliotoxicity studies. Thus, based on the results of the present study it is possible to classify nasal absorption enhancers in different categories according to their safety profile.

NOTATIONS

FITC = fluorescein isothiocyanate, HPβCD = hydroxypropyl-β-cyclodextrin, DMβCD = dimethyl-β-cyclodextrin, STDHF = sodium taurodihydrofusidate, RAMEB = randomly methylated β-cyclodextrin, SGC = sodium glycocholate, L-9 = laureth-9, LPC = L-α-lysophosphatidylcholine, LSD = Least Significant Difference.

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