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Acute histopathological effects of benzalkonium chloride and absorption enhancers on rat nasal epithelium in vivo

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Abstract

The aim of the study was to determine the acute effects of nasal absorption enhancers on the morphology of the rat nasal epithelium in vivo. Examples of three classes of enhancers were studied: the cyclodextrins dimethyl-β-cyclodextrin 2% (w/v) and randomly methylated-β-cyclodextrin 2% (w/v), the bile salt sodium glycocholate 1% (w/v), and the phospholipid L-α-lysophosphatidylcholine 1% (w/v). The preservative benzalkonium chloride 0.01% (w/v) was investigated for reasons of comparison. The compounds were dissolved in physiological saline (0.9% NaCl) and administered intranasally to anaesthetized rats. After 15 min the nasal cavity was fixated with Bouin and the tissue was processed for light microscopic examination. Morphological changes induced by physiological saline were graded as negligible or minor, and those of benzalkonium chloride as minor to major. The effects were mainly located in the respiratory epithelium. The effects of randomly methylated β-cyclodextrin and dimethyl-β-cyclodextrin were also minor and comparable to those of physiological saline. After administration of sodium glycocholate, large amounts of mucus were discharged from goblet cells and pyknosis occurred. L-α-lysophosphatidylcholine resulted in disruption of the epithelium, and damage was found not only in the respiratory epithelium but also in the olfactory epithelium. In conclusion, the acute effects of methylated β-cyclodextrins on the nasal epithelium morphology are relatively mild, and comparable to or less than those of the preservative benzalkonium chloride. Sodium glycocholate shows irreversible damage, and L-α-lysophosphatidylcholine is the most damaging absorption enhancer.

Keywords: Nasal absorption enhancers; Histology; Benzalkonium chloride; Cyclodextrins; Sodium glycocholate; L-α-lysophosphatidylcholine

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1. Introduction

The use of absorption enhancers in nasal delivery of peptide and protein drugs is often indispensable (Lee et al., 1991). The effects and mechanism of action of absorption enhancers are still under investigation, especially in relation to the potentially damaging side effects of nasal absorption enhancers, which have yet to be characterised. For clinical use of nasal peptide drug formulations, it is necessary that the interaction of nasal peptide drug formulations with the nasal epithelium is well understood and that their safety can be guaranteed.

Several classes of substances are used as nasal absorption enhancers, e.g. cyclodextrins, bile salts and phospholipids. From these three groups the following enhancers were chosen to compare their acute histological effects: the cyclodextrins dimethyl-β-cyclodextrin and randomly methylated β-cyclodextrin, the bile salt sodium glycocholate, and the phospholipid L-α-lysophosphatidylcholine. The effects of absorption enhancers on the nasal epithelium have been studied using several models. One of these models, the ciliary beat frequency (CBF), studies the in vitro effects of substances on the CBF of newborn chicken trachea (van de Donk et al., 1980, 1982). Secondly, in vivo effects of enhancers on the nasal epithelium have been investigated by determining the release of marker compounds from the rat nasal cavity, after administration of an absorption enhancer (Marttin et al., 1995). For sodium glycocholate and L-α-lysophosphatidylcholine histological and clinical data are available which indicate that they can have damaging effects (Pontiroli et al., 1989, 1991; Donovan et al., 1990; Ennis et al., 1990; Chandler et al., 1991b). However, there are no morphological data on the influence of methylated β-cyclodextrins on the nasal epithelium.

Therefore, in this study the acute effects of a single nasal administration of absorption enhancers of several classes, including the methylated β-cyclodextrins, on the morphology of the rat nasal epithelium were investigated. For comparison the acute histological effects of the preservative benzalkonium chloride were studied, because this compound is an additive of numerous and widely used nasal drug formulations.

2. Materials and methods

2.1. Materials

Hypnorm (containing 0.315 mg fentanyl citrate and 10 mg fluanisone per ml 0.9% NaCl) was acquired from Janssen Pharmaceutical (Oxford, United Kingdom) and Nembutal (sodium pentobarbital, 60 mg/ml) from Sanofi B.V. (Maassluis, The Netherlands). Benzalkonium chloride (BAC) was obtained from Brocacef (Maarssen, The Netherlands). Dimethyl-β-cyclodextrin (DMβCD) was from Avebe (Foxhol, The Netherlands) and randomly methylated β-cyclodextrin (RAMEB) from Wacker (Burghausen, Germany). The degree of substitution of the methylated β-cyclodextrin derivatives was 2.0 for DMβCD and 1.8 for RAMEB. Sodium glycocholate (SGC) and L-α-lysophosphatidylcholine (LPC) were purchased from Sigma (St. Louis, MO, USA). Formaldehyde was obtained from Merck (Darmstadt, Germany), picric acid for microscopy from Fluka A.G. (Buchs, Germany) and acetic acid 98% from J.T. Baker (Deventer, The Netherlands).

2.2. Methods

2.2.1. Preparation of solutions

All solutions were prepared in physiological saline (0.9% NaCl), in Millipore water. The following concentrations were studied: BAC 0.01% (w/v), DMβCD 2% (w/v), RAMEB 2% (w/v), SGC 1% (w/v) and LPC 1% (w/v). These concentrations are based on those frequently used in nasal drug delivery studies in animals and man. Each formulation was administered in five rats. Four rats received an intranasal dose of physiological saline, to measure effects due to administration of the formulation vehicle without BAC or any absorption enhancer.

2.2.2. Animal preparation and dosing

Male Wistar rats (Broekman Instituut B.V., Someren, The Netherlands), weighing between 225 and 275 g, were used. Animals were anaesthetized with Hypnorm (0.8 ml/kg) subcutaneously and with Nembutal (0.33 ml/kg) intraperitoneally. A 5 cm silicone cannula was inserted into the trachea via trachectomy to enable breathing. A second cannula of 20 cm silicone was inserted via an incision in the oesophagus into the posterior end of the nasal cavity, to perfuse the fixative and to prevent drainage of the formulation from the nasal cavity. The intranasal dosage was applied unilaterally into the right nostril. Dosages were administered using a 50 μl Hamilton syringe with a PVC-tube attached. The tube was inserted at least 0.5 cm into the nostril, and a small volume (20 μl) was administered to the nasal cavity. This small volume was chosen to avoid leaking of the solution to the left side of the nasal cavity; this side could then be used as an undosed control (Chandler et al., 1991a). During dosing and 15 min exposure time to the absorption enhancers rats were in supine position. Previous histological studies of nasal absorption enhancers have shown that after an exposure time of 15 min the acute effects of the enhancer are manifest (Ennis et al., 1990; Chandler et al., 1991a). Moreover, under physiological circumstances the mucociliary clearance removes substances from the nasal cavity to the oesophagus in about 10–15 min. Before the end of the allowed exposure time, rats were deeply anaesthetized with an intraperitonal or intravenous injection of 0.5 ml Nembutal. Fixation of the tissue was started exactly 15 min after administration of the nasal formulation.

2.2.3. Fixation, decalcification and embedding for light microscopy

The nasal cavity of the rats was flushed with 5 ml freshly prepared Bouin's fixative (saturated picric acid: formaldehyde solution: acetic acid = 15:5:1 v/v/v) via the oesophageal cannula. After perfusion fixation the rats were decapitated and the mandibles, skin and soft tissue were removed from the skull. The heads were subsequently immersed in fresh fixative for 24 h. Heads were rinsed in 50% ethanol and stored in 70% ethanol. Decalcification took place in formic acid-based decalcifation solution for 7 days. The nose was cut into five cross-sections with a scalpel, cutting the first one right before the upper incisor teeth and the next one right behind them. The following two cross-sections were cut in the area between the incisor teeth and the eyes. The last cross-section was cut right in front of the eyes. The cross-sections were numbered from 1 to 4 consecutively, starting with the first cross-section behind the incisor teeth. The cross-section in front of the incisor teeth was not numbered because no effects of all the treatments were observed in this section. All cross-sections were dehydrated through graded alcohols and embedded in paraffin. Each cross-section was oriented so that complete transverse cross-sections of the nasal cavity were obtained. Sections of a thickness of 5 μm were cut using standard procedures. Each cross-section was stained with haematoxylin/eosin (HE) and periodic acid Schiff (PAS, for mucosubstances).

2.2.4. Light microscopic examination and scoring of effects

Cross-sections of the nasal cavity were examined with a light microscope (Olympus, Japan). All slides were coded to enable blind scoring. The left side of the nasal cavity was used as a control, and tissue morphology of the dosed side was compared with the undosed side (Uraih and Maronpot, 1990). Sections of animals receiving physiological saline were checked for effects of the formulation vehicle. Sections of the nasal cavity were examined at enlargements of 40, 100, 250, 400 and 1000 $\times$.

Five rats were treated with benzalkonium chloride and each enhancer. The physiological saline solution was administered to four rats. For each rat the four cross-sections behind the upper incisor teeth were scored. The effects of the enhancers as compared to the untreated side were scored per cross-section. The morphology of the epithelium of the nasal cavity was evaluated and scored for the following five criteria: (1) ciliary integrity, (2) apical cell border integrity, (3) mucus extrusion, (4) pyknosis of cell nuclei and (5) epithelial disruption. Changes in the ciliary and the

apical cell border integrity ranged from barely visible dishevelment of cilia, which was scored as a minor effect, to obvious cluttering and disarrangement of cilia, scored as a major effect. The most severe score for effects on cilia was the absence of cilia. Major effects on the apical cell border were observed as swelling of the apical cell border, giving it an irregular appearance. Mucus extrusion was recorded using PAS stained slides, registering the amount of mucus in the nasal cavity and the number of goblet cells filled with mucus. The score ranged from no change, with all goblet cells filled with mucus, to complete extrusion of mucus in the nasal cavity. Pyknosis is the shrinking of cell nuclei and is indicative of necrosis. The process of pyknosis is irreversible, and could be recognized by darker colouring of the nucleus and shrinkage. The last criterium was epithelial disruption. In that case, the basal lamina was completely denuded of columnar cells in particular places, leaving a layer of flat basal cells on the septum. The disrupted cells were dislodged in the nasal cavity. The nuclei of unattached cells were not pyknotic, but in the same segment pyknosis was observed in the cells still attached to the basal lamina. The grading of the severity of the epithelial damage was based on the scores of the five criteria (see Table 1). The grade 'minor effect' was assigned to treatments leading to slight mucus extrusion and minor changes to the ciliary and apical cell border integrity. 'Major damage' was graded when large mucus discharge and major changes to ciliary and cell border integrity were observed after administration of the substances. Because pyknosis only occurred in epithelia that showed extensive irregularity of the cilia and deformation of the apical cell border, or even complete loss of cilia and abundant to complete mucus discharge, it was graded as 'irreversible changes'. For disruption of the epithelium, which was the most serious effect observed, the grade 'severe damage' was given. An average grading of the cross-sections 1–4 was determined for each rat.

For each solution the localisation of the effects on the epithelium of the nasal cavity was also established. Lesions of the nasal epithelium and the emptying of goblet cells in the nasal cavity were recorded to map the distribution of the nasally administered absorption enhancers and to identify the susceptibility of different epithelial cell types to the different enhancers. Localisation of the lesions was recorded using diagrams of the nasal passages of L-344 rats (Méry et al., 1994).

3. Results

3.1. Morphology of the untreated side of the nasal cavity

The untreated side of the nasal cavity was not affected by administering a solution containing BAC or enhancers to the contralateral side of the cavity. Therefore, the untreated side of the nasal cavity was consistently used as the control side. Only for LPC treated rats, damage was sometimes observed in the untreated side; in these cases the general appearance of the untreated side of other samples was used as the control.

Table 1
Histological scoring

Grading		Description
No change	–	No changes
Minor changes	+	Minor changes cilia Minor changes apical cell border integrity Slight mucus discharge
Major changes	+ +	Disarrangement of cilia Major changes apical cell border integrity Abundant mucus discharge
Irreversible changes	+ + +	Irregularity or complete loss of cilia Severe deformation of apical cell border Abundant to complete mucus discharge Pyknosis
Severe damage	+ + + +	Complete loss of cilia Severe deformation of apical cell border Complete mucus discharge Pyknosis Epithelial disruption

In the untreated side of the nasal cavity the following morphology of cells was observed. The cross-section in front of the incisor teeth contained squamous epithelium. Because it was not affected by the solutions studied due to the keratinised structure of the squamous epithelium, this section was not scored. The septum of the first and second cross-sections behind the incisor teeth was covered with pseudo stratified columnar ciliated epithelium and goblet cells, forming the respiratory epithelium (Fig. 1a). The lining of the cilia was observed to be a straight line, and the cell membranes of these cells and goblet cells were also straight lines. On the basolateral side of the epithelium basal cells were found. In PAS coloured slides mucus was clearly visible in goblet cells and on the apical side of the epithelium (Fig. 1b). In the third and fourth cross-sections the septum was covered with columnar cells, but goblet cells were not present (Fig. 1c). On the dorsal part of the septum and on the dorsal meatus the olfactory epithelium was located. The olfactory epithelium was covered with microvilli and a layer of mucus (Fig. 1d).

3.2. Effects of BAC and absorption enhancers on the nasal epithelium

The histopathological scoring of sections is explained in Table 1. The effects of the formulation vehicle (physiological saline) were negligible when compared to the untreated side (Table 2). The cilia on the cell membrane sometimes looked slightly dishevelled, i.e. they were not as straight as those in the untreated side. Apical cell border integrity was sometimes slightly affected, and a small amount of mucus was occasionally extruded.

For the preservative BAC in one rat no changes in comparison to the untreated side were observed, while in one rat pyknosis was observed (Table 2). The first to third cross-sections showed major changes in ciliary and apical cell border appearance

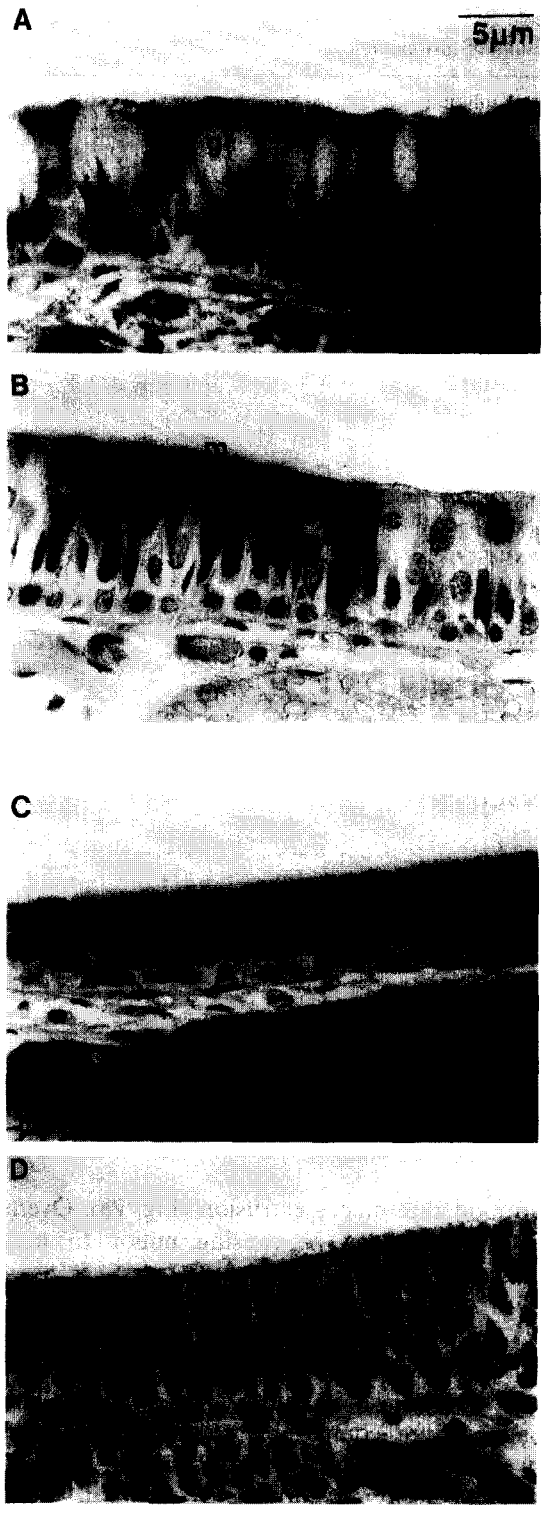

Fig. 1. Untreated rat nasal epithelium. (a) Respiratory epithelium (1000 × enlargement, HE staining); (b) respiratory epithelium (1000 × enlargement, PAS staining); (c) respiratory epithelium (1000 × enlargement, HE staining); (d) olfactory epithelium (1000 × enlargement, HE staining); b, basal cell; c, ciliated cell; g, goblet cell; lm, basal lamina; m, mucus; o, olfactory cell.

Table 2
Results of histopathology

Nasal solution	Cross-sections				Average grading (according to Table 1)
	1	2	3	4	
NaCl 0.9%	–	–	+	–	No change
NaCl 0.9%	+	+	+	–	Minor changes
NaCl 0.9%	+	–	–	+	No to minor changes
NaCl 0.9%	–	+	–	–	No change
Preservative					
BAC 0.01%	++	++	+	+	Minor to major changes
BAC 0.01%	+++	++++	+	+	Minor to severe damage
BAC 0.01%	++	++	++	–	Major changes
BAC 0.01%	–	–	–	–	No change
BAC 0.01%	++	+	–	–	Minor changes
Enhancer					
RAMEB 2%	+	+	+	–	Minor changes
RAMEB 2%	+	+	+	–	Minor changes
RAMEB 2%	+	+	+	–	Minor changes
RAMEB 2%	+	+	–	–	No to minor changes
RAMEB 2%	–	–	–	–	No change
DMβCD 2%	+	+	+	+	Minor changes
DMβCD 2%	+	+	+	–	Minor changes
DMβCD 2%	+++	+	+	+	Minor to major changes
DMβCD 2%	+	+	+	+	Minor changes
DMβCD 2%	+	+	–	–	No to minor changes
SGC 1%	++++	++++	++++	++	Severe damage
SGC 1%	+++	+++	+++	++	Irreversible changes
SGC 1%	+++	++++	+++	+++	Irreversible changes
SGC 1%	+++	+++	+++	+++	Irreversible changes
SGC 1%	+++	+++	+++	+	Irreversible changes
LPC 1%	++++	++++	++++	++++	Severe damage
LPC 1%	++++	++++	++++	++++	Severe damage
LPC 1%	++++	++++	++++	+++	Severe damage
LPC 1%	++++	++++	++++	++++	Severe damage
LPC 1%	++++	++++	++++	++++	Severe damage

and moderate mucus extrusion (Fig. 2a). Overall, BAC was graded as causing minor to major changes.

For the methylated β-cyclodextrin RAMEB (2% w/v) in 0.9% NaCl minor changes in ciliary and apical cell border integrity were found in the first, second and third cross-section. These changes were comparable to those induced by physiological saline without RAMEB. However, changes were observed more frequently than with physiological saline, and slight mucus extrusion also occurred (Table 2). The effects of RAMEB were localised on medial and dorsal parts of the septum where the respiratory epithelium is found (Fig. 2b). The overall effects of RAMEB on the nasal epithelium were graded as minor effects. For the methylated β-cyclodextrin DMβCD (2% w/v) in 0.9% NaCl minor changes were found occasionally in the fourth cross-section, but mostly in cross-sections 1–3 where minor changes to ciliary and apical cell border integrity were observed (Fig. 2c). The extrusion of mucus was

scored as slight to moderate. In one rat major changes in ciliary and apical cell border integrity occurred.The localisation of the effects was comparable to the effects of RAMEB. Overall effects of DMβCB were graded as minor, also comparable to RAMEB (Table 2). Compared to BAC, the effects of RAMEB and DMβCD were smaller, but they were more pronounced than those of physiological saline alone.

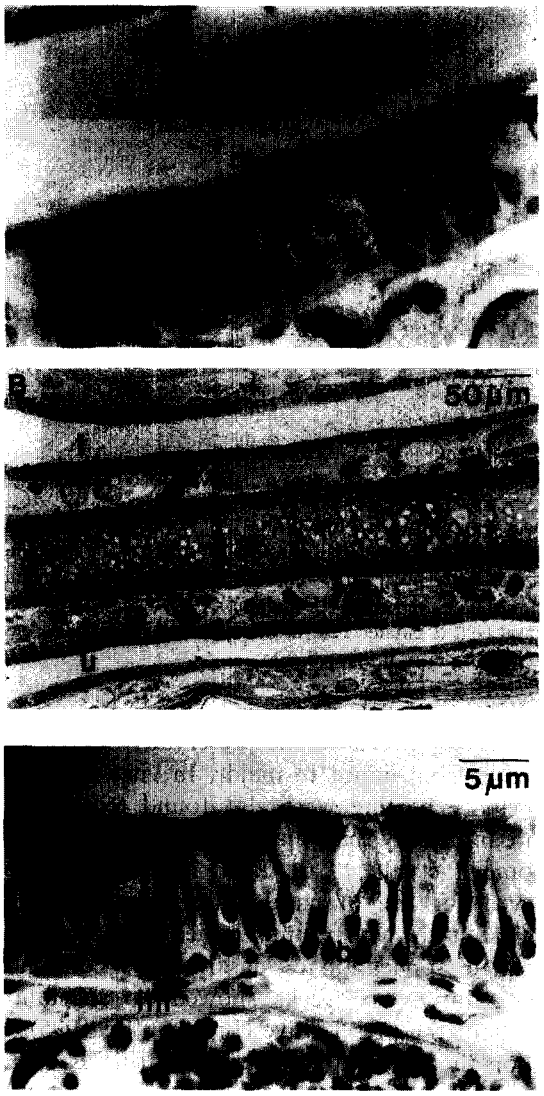

Fig. 2. Respiratory epithelium showing minor effects after nasal administration of solutions. (a) Treated with BAC 0.01% (1000 × enlargement, PAS staining); (b) treated with RAMEB 2% (100 × enlargement, PAS staining); (c) treated with DMβCD 2% (1000 × enlargement, HE staining (1); b, basal cell; c, ciliated cell; g, goblet cell; lm, basal lamina; m, mucus; s, septum; t, treated side; u, untreated side.

The bile salt enhancer SGC 1% (w/v) in physiological saline appeared to exhibit a strong damaging effect. The first to third cross-sections showed a number of toxic effects. Obvious distortion of the cilia and loss of cilia occurred, while the apical cell border was deformed. Pyknosis was seen in all rats studied (Fig. 3a). Abundant to complete mucus extrusion was also apparent (Fig. 3b). In one rat epithelial disruption was found. The fourth cross-section was also severely affected, but pyknosis did not always occur. The overall grading for this enhancer was designated as 'irreversible changes' (Table 2). In contrast to the methylated cyclodextrins and BAC, the effects of SGC were damaging and the lesions were more widespread within the nasal cavity. They were recorded on the septum (both medial and dorsal part), the medial part of the meatus, the dorsal scroll of the nasoturbinate, and the medial part of the first ethmoturbinate. Therefore, SGC affected not only the respiratory epithelium but also cells of the olfactory epithelium, although the effects were most prominent in the respiratory area.

After intranasal administration of the phospholipid enhancer LPC 1% (w/v) in physiological saline severe damage occurred in all cross-sections: cilia were irregular or absent and the apical cell border was deformed (Fig. 3c). In the first to third cross-sections abundant to complete mucus extrusion occurred, and pyknosis and epithelial disruption were seen. On particular parts of the septum only the basal lamina was visible, because the epithelium was completely detached. LPC was graded as causing severe epithelial damage. It also gave the most widespread localisation of damage. The lesions were not only present on the medial and dorsal part of the septum, but also occasionally on the superior ventral medial part. The medial, middle lateral and dorsal parts of the meatus and even the medial and lateral part of the first ethmoturbinate were affected. LPC affected several epithelial cell types, i.e. respiratory and olfactory epithelia (Fig. 3d).

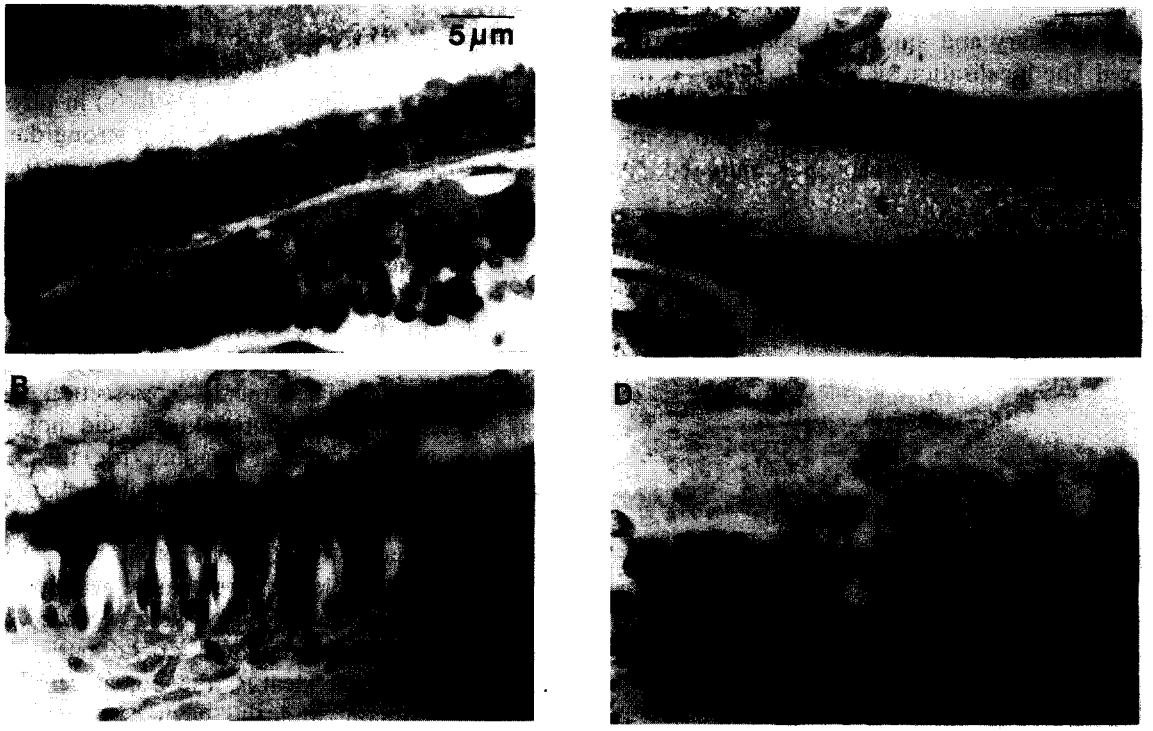

Fig. 3. Nasal epithelium showing irreversible or severe damage after nasal administration of solutions. (a) Respiratory epithelium treated with SGC 1%, pyknosis is visible by the shrinking of cell nuclei (1000 × enlargement, HE staining); (b) respiratory epithelium treated with SGC 1%, showing mucus discharge from goblet cells (1000 × enlargement, HE staining); (c) respiratory epithelium treated with LPC 1%, epithelium disruption is visible at the treated side (100 × enlargement, HE staining); (d) olfactory epithelium after treatement with LPC 1% (1000 × enlargement, HE staining); b, basal cell; c, ciliated cell; d, disrupted epithelium; lm, basal lamina; m, mucus; o, olfactory cell; p, pyknotic nucleus; s, septum; t, treated side; u, untreated side.

4. Discussion

In the present study it was found that the nasally administered preservative BAC and absorption enhancers affected mainly the respiratory epithelium, which is distributed over the septum and the dorsal meatus. This localisation of effects might be contributed to the dorsal position of the rat during the administration and incubation of the solution. Lesions of the olfactory epithelium were only seen after administration of SGC (1%) and LPC (1%). The physiological saline solution elicited no or minor effects on ciliary and apical cell border appearance, and occasionally a slight release of mucus was observed.

The administration of the formulation vehicle gave rise to minor effects which add to the effects of the enhancers and preservative studied. The histological effects of the preservative BAC were ranging from minor to major. In vitro its effects on ciliary beating were also found to be much more inhibitory than those of physiological saline (Romeijn et al., 1996). BAC is world-wide approved for use in nasal drug formulations. A clinical study with rhinitis patients reported that repeated application of nasal sprays containing BAC, during six weeks showed no substantial effects of the preservative (Braat et al., 1995).

For the methylated β-cyclodextrins RAMEB (2%) and DMβCD (2%) minor effects were observed on ciliary and apical cell border appearance and moderate mucus discharge. RAMEB and DMβCD showed no significant histological differences, and their effects were comparable to or less than those of BAC. The effect of these cyclodextrins on ciliary beat frequency is also

minor, and less inhibitory than BAC 0.01% (w/v) (Romeijn et al., 1996). This is in agreement with the absence of side effects in women using a female steroid nasal formulation containing 2% DMβCD for 6 months (Hermens et al., 1991).

In studies that investigated the effect of 2 and 5% DMβCD following in situ perfusion in rats, release of intracellular and membrane bound enzymes was observed (Shao et al., 1992; Krishnamoorthy et al., 1995). However, the relevance of these observations for nasal membrane integrity is questionable. The in situ perfusion model has been developed and validated to perform absorption studies, but not for toxicological evaluation (Hirai et al., 1981). Hence, in situ perfusion of excessive volumes through the nasal cavity for periods up to 2 h is very extreme and not comparable to circumstances at which nasal drug delivery occurs. For instance, during in situ perfusion (Krishnamoorthy et al., 1995) 5 ml of a solution containing 100 mg DMβCD (2%, w/v) was perfused through the rat nasal cavity, which has a nasal volume of approximately 0.4 ml (Gizurarson, 1993). When these values are extrapolated to humans, who have a nasal volume of circa 20 ml, this would mean a perfusion with 250 ml solution containing 5 g of DMβCD. This is in sharp contrast with the nasal dose volume of 100 μl that patients generally receive, which at a concentration of 2% (w/v) would result in administration of 2500 times less, i.e. 2 mg DMβCD. Moreover, no data were given on the effects of perfusion of only the administration vehicle nor on the effects of well-known mucotoxic enhancers. Since these data are not available, it is impossible to relate the results of in situ perfusion experiments into perspective with other studies. From the present histological and previous CBF and marker compound release studies it is clear that the acute effects of methylated β-cyclodextrins on the nasal epithelium are relatively mild (Marttin et al., 1995; Romeijn et al., 1996).

Nasal administration of SGC 1% in this study resulted in irreversible and widespread changes of the epithelium. Histological studies using electron microscopy showed that the effects of SGC 1% were more seriously damaging after a 5 min incubation than those of the buffer control (Ennis et al., 1990). A light microscopic study examined the effects of SGC 1% coadministrated with PEG-600, and it was concluded that PEG-600 with SGC 1% gave rise to slight histological changes of the epithelium covering the turbinates (Donovan et al., 1990). For CBF measurements the effects of SGC are highly concentration-dependent, giving complete and irreversible ciliostasis in 20 min at a concentration of 1.5%, but not at 0.3 and 1% (Merkus et al., 1993). In human studies of nasal peptide drug formulations nasal irritation has been reported after single and repeated administration of calcitonin with 1.5% SGC (Pontiroli et al., 1989, 1991).

The most damaging enhancer studied was LPC 1% (w/v), resulting in widespread lesions over the nasal cavity, affecting the respiratory and olfactory epithelia. In another histological study LPC 0.625% was administered to the rat nasal cavity in a solution with insulin (Chandler et al., 1991b). The effects of insulin on the nasal epithelium were small, whereas the effects of insulin with LPC were very damaging, leading to complete mucus extrusion and epithelium disruption. These results are in accordance with the morphological effects of LPC found in this study. In CBF studies LPC 1% was also rated as a very damaging enhancer, causing irreversible ciliostasis within 5 min (Merkus et al., 1993).

The histological results are also in agreement with data from in vivo studies measuring the release of marker compounds in the nasal cavity (Marttin et al., 1995). Enhancers were grouped according to their effects on the release of marker compounds, placing the methylated β-cyclodextrins in the least damaging group, SGC in an intermediate group and LPC in the most damaging one. The same rank order has also been found in the present histological study. In the in vivo release study LPC was the only enhancer that released the intercellular protein acid phosphatase, an enzyme mainly found in olfactory cells (Randall et al., 1987). Similarly, in this histological study LPC was also shown to be the most damaging enhancer for olfactory cells.

In conclusion, the acute histopathological effects of physiological saline (NaCl 0.9%) and of methylated β-cyclodextrins RAMEB and DMβCD on the nasal epithelium of the rat can be classified as absent or mild. These effects are comparable to or less than BAC, which is ap-

proved for use as a preservative in human nasal drug formulations. The acute effects of SGC 1% on the nasal epithelium of the rat are found to be damaging and irreversible. The use of the bile salt SGC as an absorption enhancer in nasal drug formulations is questionable, based on the results of this study and the reports of irritating side effects in clinical studies. The phospholipid LPC 1% appeared to be the most damaging absorption enhancer, probably prohibitive for clinical use.

References

Braat, J.P.M., Ainge, G., Bowles, J.A.K. et al., The lack of effect of benzalkonium chloride on the cilia of the nasal mucosa in patients with perennial allergic rhinitis: a combined functional, light, scanning and transmission electron microscopy. *Clin. Exp. All.*, 25 (1995) 957–965.

Chandler, S.G., Illum, L. and Thomas, N.W., Nasal absorption in the rat. I: A method to demonstrate the histological effects of nasal formulations. *Int. J. Pharm.*, 70 (1991a) 19–27.

Chandler, S.G., Illum, L. and Thomas, N.W., Nasal absorption in rats.II. Effect of enhancers on insulin absorption and nasal histology. *Int. J. Pharm.*, 76 (1991b) 61–70.

van de Donk, H.J.M., Zuidema, J. and Merkus, F.W.H.M., The influence of pH and osmotic pressure upon tracheal ciliary beat frequency as determined with a new photoelectric registration device. *Rhinol.*, 18 (1980) 93–104.

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. *Rhinol.*, 20 (1982) 81–87.

Donovan, M.D., Flynn, G.L. and Amidon, G.L., The molecular weight dependence of nasal absorption: the effect of absorption enhancers. *Pharm. Res.*, 7 (1990) 808–815.

Ennis, R.D., Borden, L. and Lee, W.A., The effects of permeation enhancers on the surface morphology of the rat nasal mucosa: A scanning electron microscopy study. *Pharm. Res.*, 7 (1990) 468–475.

Gizurarson, S., The relevance of nasal physiology to the design of drug absorption studies. *Advan. Drug Deliv. Rev.*, 11 (1993) 329–347.

Hermens, W.A.J.J., Belder, C.W.J., Merkus, J.M.W.M. et al., Intranasal estradiol administration to oophorectomized women. *Eur. J. Obs. Gynecol. Reprod. Biol.*, 40 (1991) 35–41.

Hirai, S., Yahiki, T., Tai, M. and Mima, H., Absorption of drugs from the nasal mucosa of rat. *Int. J. Pharm.*, 7 (1981) 317–325.

Krishnamoorthy, R., Wolka, A.M., Shao, Z. and Mitra, A.K., Cyclodextrins as mucosal absorption promoters IV. Evaluation of nasal mucotoxicity. *Eur. J. Pharm. Biopharm.*, 41 (1995) 296–301.

Lee, V.H.L., Yamamoto, A. and Bhaskar Kompella, U., Mucosal penetration enhancers for facilitation of peptide and protein drug absorption. *Crit. Rev. Ther. Drug Carr. 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. Control. Rel.*, 24 (1993) 201–208.

Méry, S., Gross, E.A., Joyner, D.R., Godo, M. and Morgan, K.T., Nasal diagrams: a tool for recording the distribution of nasal lesions in rat and mice. *Toxicol. Pathol.*, 22 (1994) 353–372.

Pontiroli, A.E., Alberetto, M., Calderara, A., Pajetta, E. and Pozza, G., Nasal administration of glucagon and human calcitonin to healthy subjects: a comparison of powders and spray solutions and of different enhancing agents, *Eur. J. Clin. Pharmacol.*, 37 (1989) 427–430.

Pontiroli, A.E., Pajetta, E., Calderara, A. et al., Intranasal and intramuscular human calcitonin in female osteoporosis and in Paget's disease of bones: a pilot study, *J. Endocrinol. Invest.*, 14 (1991) 47–51.

Randall, H.W., Bogdanffy, M.S. and Morgan, K.T., Enzyme histochemistry of the rat nasal mucosa embedded in cold glycol methacrylate, *Am. J. Anat.*, 179 (1987) 10–17.

Romeijn, S.G., Verhoef, J.C., Marttin, E. and Merkus, F.W.H.M., The effect of nasal drug formulations on ciliary beating in vitro, *Int. J. Pharm.*, 135 (1996) 137–145.

Shao, Z., Krishnamoorthy, R. and Mitra, A.K., Cyclodextrins as nasal absorption promoters of insulin: mechanistic evaluations, *Pharm. Res.*, 9 (1992) 1157–1163.

Uraih, L.C. and Maronpot, R.R., Normal histology of the nasal cavity and application of special techniques, *Environ. Health Persp.*, 85 (1990) 187–208.