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International Journal of Pharmaceutics 130 (1996) 93–102

international journal of pharmaceutics

Recovery of the nasal mucosa following laureth-9 induced damage

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Received 19 June 1995; revised 25 July 1995; accepted 23 October 1995

Abstract

Polyoxyethylene-9-lauryl ether (laureth-9) has been used in drug formulations for nasal delivery to promote drug absorption and increase bioavailability, especially for compounds with high molecular weights. However, it has also been reported that laureth-9 causes morphological damage to the nasal membranes when it is administered. This morphological change/damage can affect the normal functioning of the nasal epithelium, and readily increases the potential for infection and the absorption of substances through the disrupted epithelial barrier. While previous investigators have studied the mucosal repair rate from various other respiratory tissues, few reports are available regarding the repair rate of the nasal mucosa itself. Laureth-9 was used to chemically induce damage to the nasal mucosa of anesthetized rats. Individual animals were then sacrificed at various times over the following 10 days for morphological examination of the nasal mucosa. Following euthanasia, the tissues of the nasal cavity were decalcified, fixed, sectioned, and stained with hematoxylin and eosin for light microscopic examination. Histological examination of mucosal samples taken 4 h after exposure showed no apparent morphological changes, whereas samples taken 24 and 48 h after administration showed signs of severe damage to the epithelium. Regrowth of the epithelium could be observed beginning on the third day following laureth-9 exposure; there was evidence of basal cell regrowth and differentiation by the fourth day. A completely regenerated epithelium could be observed between the seventh and the tenth days following exposure to the surfactant.

Keywords: Polyoxyethylene-9-lauryl ether (laureth-9); Nasal drug delivery; Morphology; Epithelial regrowth

1. Introduction

Drug administration by the nasal route is an alternative to the parenteral route due to the relative ease of administration and rapid absorption from this site. Many compounds show a relatively high permeability through the nasal mucosa, and some peptide and protein compounds, which show very low bioavailabilities following oral administration, have greater bioavailabilities following nasal delivery. However, the mobility of large compounds through the nasal mucosa generally decreases as their molecular weight increases (Fisher et al., 1987; Maitani et al., 1989; Donovan et al., 1990). It is only in the presence of an absorption enhancer that many high molecular

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SSDI 0378-5173(95)04358-*H*

Fig. 1. Light micrograph of rat nasal cavity. A septal cross section from a control rat. Normal epithelium (magnification 1100 ×).

weight compounds show clinically useful bioavailabilities (Aungst and Rogers, 1988; Donovan et al., 1990). Compounds such as sodium lauryl sulfate, sodium glycocholate, sodium taurodihydrofusidate, DEAE-dextran, lysophosphatidylcholine, laureth-9, and bile salts have all been reported to promote drug absorption following nasal administration (Hirai et al., 1981; Chandler et al., 1991a). However, evidence from histological examinations show that some enhancers may seriously damage the nasal mucosa (Donovan et al., 1990). For example, the correlation between the increasing fraction of insulin absorbed using various types of enhancers has been studied. The results indicated a close relationship between increasing insulin absorption and the degree of epithelial damage observed. Among the enhancers studied, 1% laureth-9 induced the most severe damage to the nasal mucosa (Chandler et al., 1991a).

The morphologic changes in the nasal mucosa caused by drugs, enhancers, or other formulation additives, may result in damage to the ability of the nasal mucosa to carry out its natural defense functions. In addition, chronic infection may occur when recovery or regeneration of the normal nasal epithelium cannot be achieved (Boling, 1935). This may lead to chronic inflammation of the nasal tissues or to other infectious diseases, such as rhinoscleroma. Thus, it is important to investigate the recovery rate of the nasal mucosa following chemically induced damage to limit the possibility of chronic drug- or formulation-induced damage.

Previous investigators have studied the regeneration of the tracheal mucosa in several species including rat, rabbit, dog and calf (Adams et al., 1930; Wilhelm, 1953; Hilding, 1965; Nordin, 1982). These studies indicated that the time for recovery of the damaged epithelium ranged from days to months depending on the method of injury and the extent of damage. McGregor (1931) studied the regeneration of the mucous membrane in the human antrum. Regrowth of a

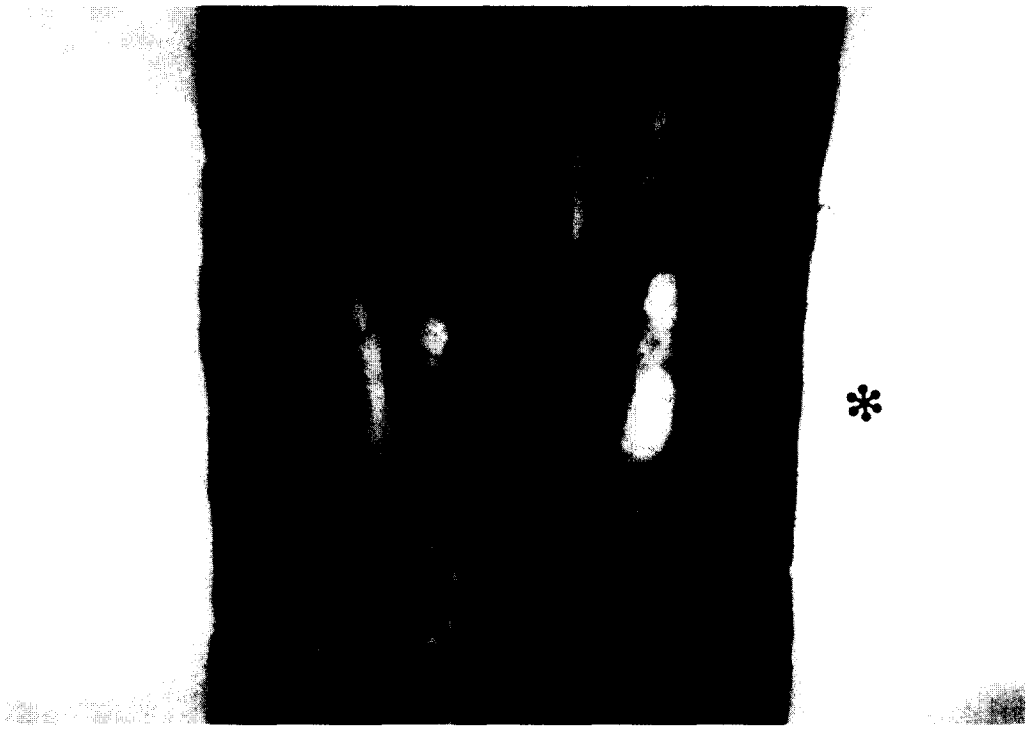

Fig. 2. Light micrograph of a septal cross section 4 h following exposure to 1% laureth-9. No obvious changes in the epithelium can be seen on the dosed side (*) (magnification 1100 ×).

columnar epithelium six months after removal of the antral epithelium was observed. Coates and Ersner (1930) observed that the mucosal membrane in the frontal sinus of dogs was regenerated 2 months after surgical removal of the epithelium. Regeneration of the nasopharyngeal epithelium in the monkey was studied by Leela et al. (1975) following a cotton swab injury. They described the regeneration of an epithelium containing columnar ciliated cells interspersed with typical goblet cells five days after the lesion was induced.

Considerably less attention has been paid to the regeneration of the nasal epithelium. Boling (1935) was the first investigator to study the recovery of the nasal mucosa instead of the sinus mucosa. In his studies, mechanical injury was induced using a modified curet. This tool consisted of a sharp scraping blade preceded by two parallel knives at each side, such that the mucosa was excised by drawing the instrument forward. Results showed that the rate of complete recovery was dependent on the size of the injury. A complete, intact epithelium could be observed 3–8 days after injury provided there was no infection. Other investigators (Ohashi et al., 1986; Ohashi et al., 1991) also studied the regeneration of the nasal mucosa after both mechanical injury using a modified curet and chemical injury induced by exposure to styrene vapor. Recovery of the nasal mucosa in rabbits following mechanical injury demonstrated that complete recovery of the nasal mucosa was obtained within 5 days provided the basal cells and basement membrane were left intact. In comparison, it took 6 weeks for complete recovery if the entire epithelium, including the basal cells, was removed. Styrene, which has been reported to induce respiratory damage in the rat, was used to chemically initiate damage to both the nasal and tracheal epithelia. The recovery period varied from several days to several months depending on the region of the respiratory tract involved and the concentration of styrene used. Comparing the recovery results of the respiratory membrane subjected to either chemical exposure or

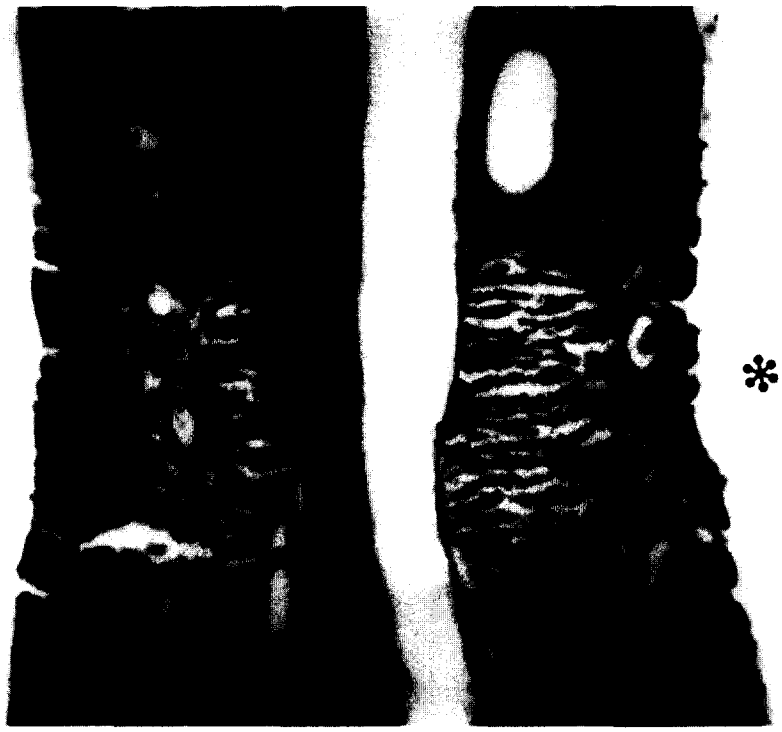

Fig. 3. Light micrograph of a septal cross section 24 h following exposure to 1% laureth-9. Dosed side (*) shows significant damage to epithelium (magnification 1100 ×).

mechanical injury, it appears that the mucosa which was damaged following exposure to styrene was not regenerated as readily as the mucosa affected by mechanical injury.

Laureth-9, which has been investigated as an enhancer in nasal formulations, has been reported to cause severe degeneration of the nasal epithelium at a concentration of 1% (Donovan et al., 1990; Chandler et al., 1991a). Human nasal irritation after exposure to several concentrations of laureth-9 in an insulin formulation was previously investigated by Salzman and his co-workers (Salzman et al., 1985). Nearly half of the patients reported discomfort with 1% laureth-9, whereas most tolerated concentrations of 0.25%, and all tolerated a concentration of 0.1%.

As a result of these observations, 1% laureth-9 was selected as an agent to induce slight, yet repairable, damage to the nasal mucosa of rats in order to study the repair rate following exposure to a potentially damaging drug agent or formulation. Since formulations containing some absorption enhancers, e.g., laureth-9, can alter the morphology of the nasal epithelium, the use of these formulations in chronic therapies may not be possible. However, nasal delivery using these enhancers may still be a strategy for acute use or single dose therapies for some specific compounds. Therefore, investigation of the extent of initial damage and the recovery rate of the affected epithelium becomes extremely important in assuring the safety and comfort of the individuals receiving such acute treatments.

2. Materials and methods

2.1. Materials

Polyoxyethylene-9-lauryl ether (laureth-9), urethane, *para*-formaldehyde, formic acid and sodium citrate were obtained from Sigma Chemical Co. (St. Louis, MO). Ketamine (10%) was obtained from Aveco Co. (Fort Dodge, IA) and

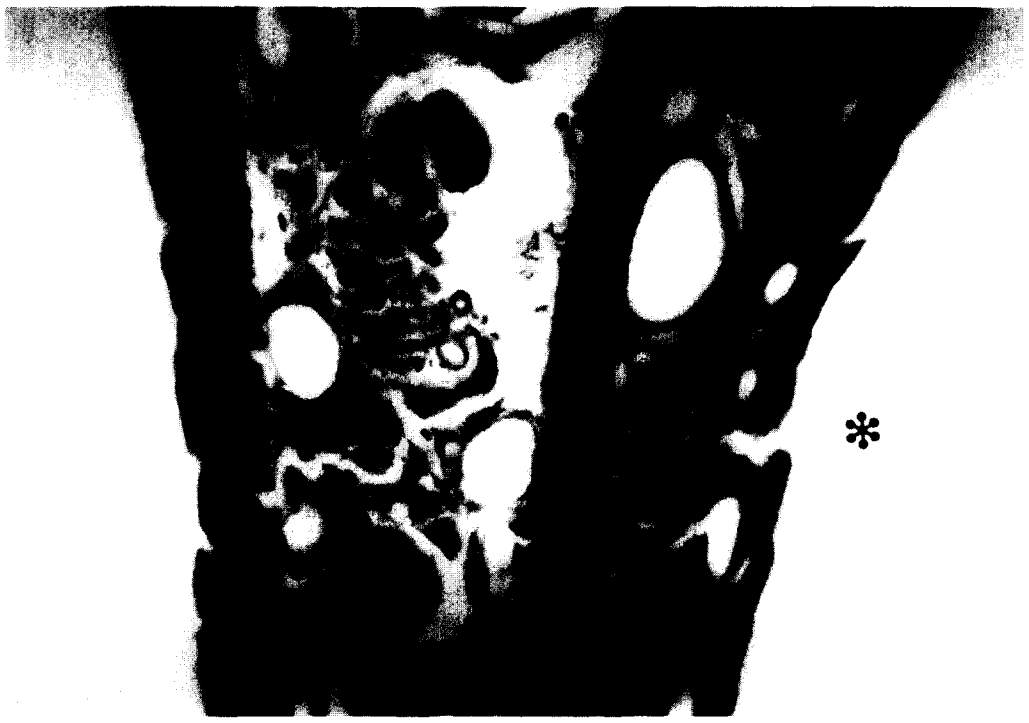

Fig. 4. Light micrograph of a septal cross section 48 h following exposure to 1% laureth-9. Dosed side (*) appears as a flattened epithelium primarily containing cuboidal cells (magnification 1100 ×).

sterile normal saline was obtained from Baxter Healthcare Corp. (Deerfield, IL). All chemicals and drug solutions were used as obtained.

2.2. Animal studies

Male, Sprague-Dawley rats (250–300 g) were housed in a virus- and antibody-free area prior to laureth-9 exposure, and in a clean, non-barrier facility following surfactant administration. Each animal was lightly sedated with 0.1–0.25 ml ketamine hydrochloride (10%) to limit struggling during dosing. Twenty-five microliters of 1% laureth-9 solution were placed into each animal's left nostril using an Eppendorf® pipette. Control animals were treated by placing 25μl of normal saline into the nostril instead of laureth-9. Mucosal samples were obtained from 2–4 rats at each time point. These animals were sacrificed with an anesthetic overdose at either 4 h or 2, 3, 4, 5, 7 and 10 days post-exposure. Samples were also obtained from one animal from a control group at each time interval.

2.3. Histology

The nasal tissues of each animal were fixed in situ by perfusing 4% *para*-formaldehyde in 0.1 M phosphate buffer solution (pH 7.4) through the circulatory system via a Teflon catheter placed into the external carotid artery from the heart. The solution was pumped at a rate of 5 ml min^{-1} using a Harvard infusion pump (Model 22, Harvard Apparatus, South Natick, MA). The nasal cavity and skull were separated, and the nasal cavity was immersed in 4% *para*-formaldehyde solution overnight. The tissues were then decalcified in a 25% formic acid/7.5% sodium citrate solution for a period of 10 days. The decalcification solution was replaced every 8–12 h. The decalcified tissues were sectioned into blocks, dehydrated in a graded series of ethanol solutions, and embedded in paraffin. Approximately 5–7 μm thick sections were cut from each block,

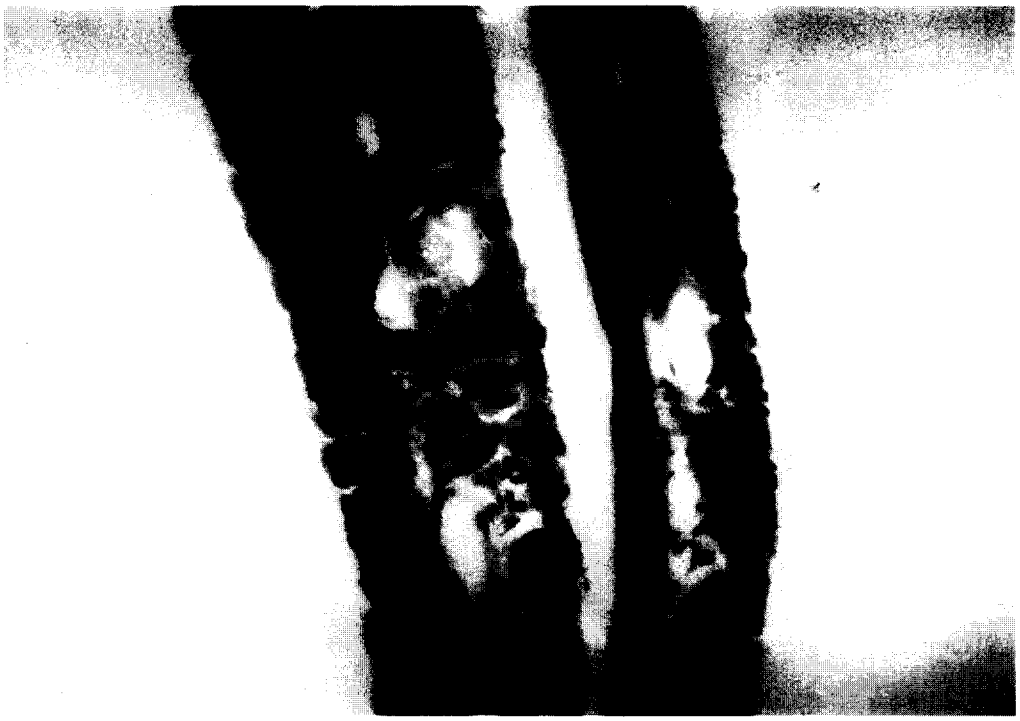

Fig. 5. Light micrograph of a turbinate cross section from the dosed nostril 72 h following exposure to 1% laureth-9. Many immature cells resulting in a loose and irregular columnar epithelium are present (magnification 1100 ×).

mounted on slides, and stained using hematoxylin and eosin. Samples of two sections from each animal were examined using light microscopy (Leitz Diaplan, Wetzlar, Germany) and photographed at a magnification of 1100 × .

3. Results and discussion

The normal nasal respiratory epithelium is a ciliated pseudostratified columnar epithelium (Fig. 1). It consists of six morphologically distinct cell types including ciliated and nonciliated columnar cells containing microvilli on their apical surfaces, goblet cells, basal cells, cuboidal cells, and brush cells (Monteiro-Riviere and Popp, 1984). The entire nasal membrane is covered by a protective mucus layer produced by the goblet cells and serous glands within the epithelium.

The patterns of degeneration and regeneration of the nasal mucosa following 1% laureth-9 exposure are shown in Figs. 2–8. The results indicate that, with the volume applied in these studies (25 μl), the undosed nostril was not affected and could be used as a control for comparison purposes. A similar observation was reported by Chandler et al. (1991b) where a volume of 20 μl was retained exclusively within the dosed nostril. Virtually no change was observed in the epithelium obtained at 4 h following exposure to a 1% laureth-9 solution (Fig. 2), although the nasal cavity itself was observed to be slightly swollen immediately after surfactant administration. Severe damage to the epithelial cells did occur, however, and shedding of the necrotic nasal epithelium was observed on the second day after exposure (Fig. 3). A similar delay in the observation of gross damage has also been reported in previous in vivo studies, where the time required to measure altered ciliary motility patterns exceeded 24 h following exposure to a potentially damaging agent (Donovan and Zhou, 1995). It is likely that this delay represents a lag time required for the cells to morphologically

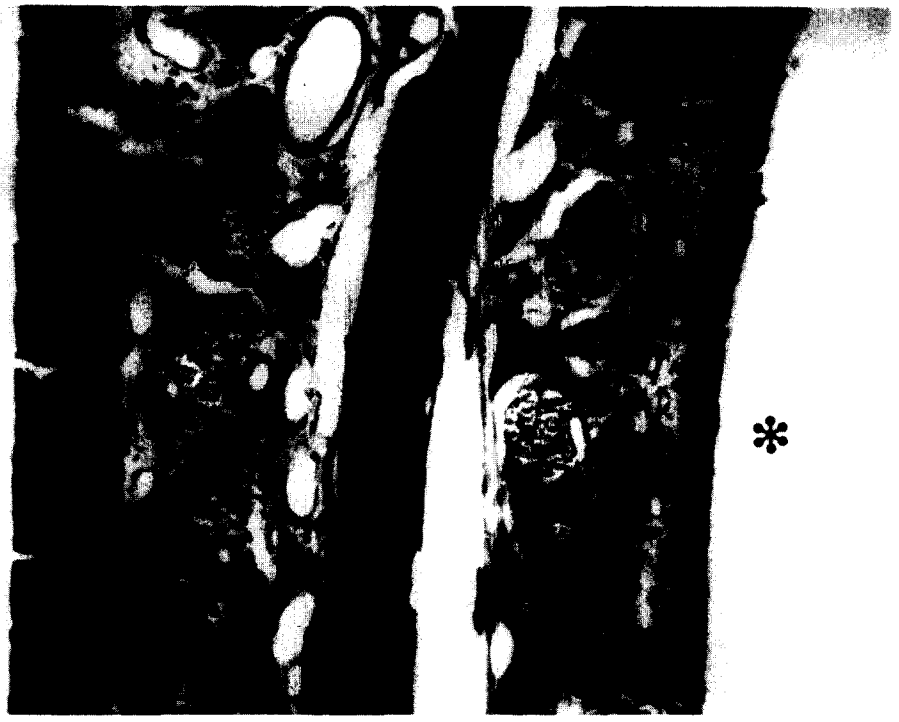

Fig. 6. Light micrograph of a septal cross section 96 h following exposure to 1% laureth-9. A short columnar epithelium is observed on the dosed side (*) (magnification 1100 ×).

manifest the damaging effects. A combination of formulation dilution in the nasal secretions and normal clearance of the damaging agent out of the nasal cavity reduce the immediate damage to the epithelium, thus requiring observation over extended time periods to fully assess the extent of morphological alteration. Other investigations have also reported that cellular detachment after cuff-induced tracheal injury occurred during the first 24 h following the injury, rather than being immediately apparent (Nordin, 1982).

On the third day following laureth-9 exposure, the regenerating epithelium appeared to primarily contain cuboidal type cells (Fig. 4). There was strong evidence for the regeneration and differentiation of the epithelium in the fourth day's results (Fig. 5). At this stage, many immature and undifferentiated cells were present, and these appear microscopically as a loose and irregular maturing columnar epithelium. Similar observations of this stage of epithelial regrowth and maturation at 24–48 h post-injury were reported following mechanical injury induced by a cotton swab to the calf trachea, and after cuff-induced injury in the rabbit trachea (Hilding, 1965; Nordin, 1982). A short columnar epithelium was observed at the fifth day post-exposure (Fig. 6). A complete columnar epithelium was formed by the seventh day post-exposure (Fig. 7). Cilia on the apical cellular surfaces were seen on the tenth day post-exposure (Fig. 8). Although it is not directly apparent by light microscopic examination whether the surfactant significantly affected the basal cells, the observation of a thinner cuboidal epithelium remaining attached to the basement membrane at days 2 and 3, along with the relatively short period of time required to replace the normal epithelium, implies that 1% laureth-9 does not cause complete loss of all of the basal cells within the epithelium. However, the damage caused by 1% laureth-9 is still of sufficient magnitude to affect some of the barrier functions of the mucosal membrane.

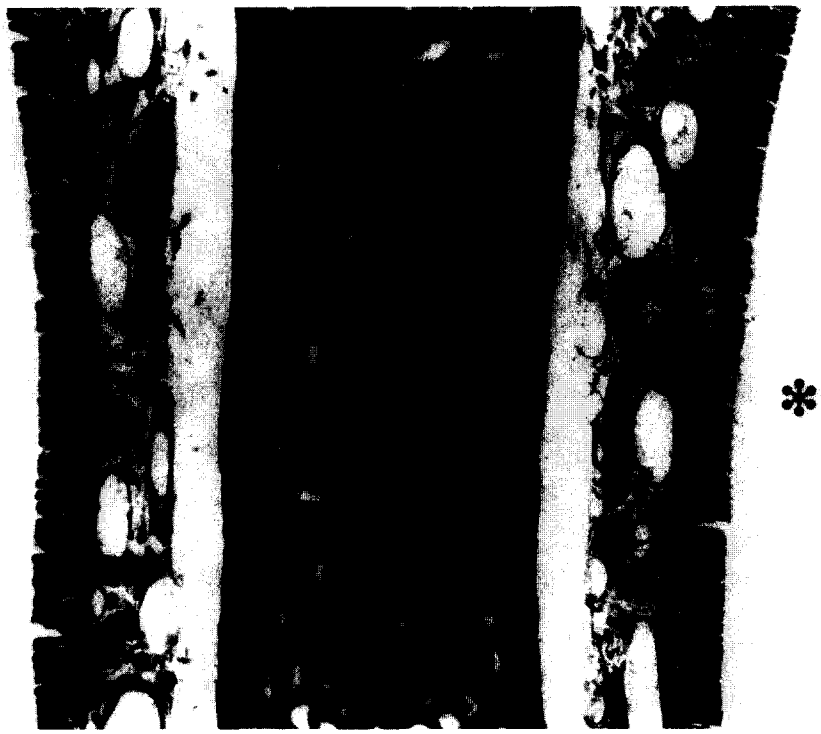

Fig. 7. Light micrograph of a septal cross section 7 days following exposure to 1% laureth-9. A normal epithelium is observed on the dosed side (*) (magnification 1100 ×).

4. Conclusions

With the increasing interest in nasal drug delivery systems, there is an associated growing concern about the extent of damage to the nasal mucosa induced by the drug itself or by formulation excipients. Some absorption enhancers have been reported to induce damage to the nasal mucosa. Among them, 1% laureth-9 has been reported to cause severe damage to the nasal mucosa. While this observation unfortunately limits the usefulness of laureth-9 as an absorption enhancer, it allows investigators to use 1% laureth-9 solutions to induce damage in the mucosa in order to study the effects of these changes on the barrier function of the nasal mucosa.

A surgically modified rat model has been commonly used to evaluate mucosal damage in the nasal cavity (Donovan et al., 1990). Surgical procedures were conducted in order to retain the injected solution in the nasal cavity throughout the entire experiment. Using this method, mucosal damage induced by 1% laureth-9 at the delivery site could be observed within several hours of initial application. This study utilized a non-surgical, open nostril rat model to more closely approximate the situation experienced in human nasal drug delivery. This non-surgical method allows for the study of the recovery rate of the damaged nasal epithelium, in addition to the observation of initial damage, since it is possible to observe morphological changes over longer time intervals (days to weeks) in comparison to the extremely short time interval (less than 6 h) available when using the surgically modified rat model. The non-surgical method provides more information regarding cellular regeneration, as well as the longer term effects of the use of nasal formulations or formulation components.

The rate of epithelial repair observed following laureth-9 induced damage is in good agreement with the repair rates found for other respiratory epithelia. Under conditions of moderate to severe damage, where the columnar epithelial cells be-

Fig. 8. Light micrograph of a septal cross section 10 days following exposure to 1% laureth-9. A normal epithelium with cilia present on the apical surface is observed on the dosed side (*) (magnification 1100 ×).

come necrotic and slough off even though some basal cells remain, complete re-epithelialization can be expected to occur within 10 days. While this rate of repair is reasonably rapid, the resulting changes in the barrier functions during this period require additional study to identify any additional risks to the individual.

Acknowledgements

This research was supported in part by the Rugby Darby Group Companies. The technical assistance provided by the staff of Central Electron Microscopy Research Facility of the University of Iowa is gratefully acknowledged.

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