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Drug effects on in vivo nasal clearance in rats

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Abstract

A simple in vivo method to study drug induced changes in nasal mucociliary clearance patterns has been developed using FluoSpheres®, fluorescent latex particles, as clearance markers in rats. The marker cleared from the nasal cavity can be collected from the nasopharyngeal area of the oral cavity and easily measured using a fluorescence spectrophotometer. Several compounds whose effects on ciliary motility have been previously studied in vitro, i.e., tripelennamine HCl, lidocaine HCl, bacitracin, neomycin sulfate, and pilocarpine nitrate, were investigated in order to validate the new in vivo technique. Clearance in rats exposed to normal saline was shown to follow a pseudo-first order pattern. Similar first order clearance patterns were observed 24 h after exposure to drug solutions which have been shown to cause reductions in ciliary beat frequency (CBF) or mucociliary clearance (tripelennamine HCl, lidocaine HCl, bacitracin) in vitro, yet the clearance rates in vivo were usually reduced. The clearance rates returned to normal within 48 h except in the case of lidocaine HCl. For compounds in which increases in CBF were observed in vitro (pilocarpine nitrate), a statistically significant increase in nasal clearance was observed 24 h after drug exposure. The ability of the in vitro method to predict the reversibility of drug induced effects was not well correlated with the results observed in vivo. It appears that homeostatic control mechanisms present in the whole animal temper some drug effects on ciliary motility, especially when morphologic changes in the cilia appear to be responsible for the reduction in CBF. This new in vivo model enables both long term changes and the time course of recovery in nasal clearance to be monitored in a single animal, thus enabling better predictions regarding drug effects on nasal clearance to be made.

Keywords: Mucociliary clearance; Nasal drug delivery; Tripelennamine; Lidocaine; Bacitracin; Neomycin; Pilocarpine; Morphology

1. Introduction

Primary ciliary dyskinesia (PCD) syndrome, a group of congenital disorders such as Kartagener's syndrome, is characterized by functional abnormalities of the cilia and subsequent impairment of normal ciliary motility patterns in the respiratory and genitourinary tracts. The dysfunctional cilia within the respiratory tract are linked to the increased occurrence of bronchiestasis, bronchial infection, and chronic rhinitis. If the application of drugs or formulation additives to the nasal mucosa results in similar patterns of ciliary dysfunction, it is likely that similar clinical pathologies could occur in chronic users of these medica-

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tions. While there are no reports of side effects of this severity occurring with the nasal or bronchial medications currently in use, the increased interest in both nasal and pulmonary drug delivery systems causes additional concern regarding the effects that the drugs themselves or their formulation additives may have on normal respiratory clearance patterns.

Cilia within the nasal cavity are present on the apical surfaces of many of the pseudostratified columnar epithelial cells of the main nasal cavity and nasopharyngeal regions. The coordinated beating of the cilia results in the movement of the overlying mucus layers towards the nasopharynx. The mucus blanket is actually composed of two distinct phases: the first, a low viscosity aqueous layer surrounding the cilia (periciliary layer) and the second, a more viscous layer containing mucopolysaccharides (mucus layer). As a result of the dynamic interactions between the beating cilia and the two phases of the mucus layers, either malfunctioning cilia or a change in the physical properties of the mucus can result in inefficient mucociliary clearance.

In order to investigate the effects of components of intranasal dosage forms on mucociliary function, various in vivo and in vitro methods have been developed. Several authors have provided excellent reviews of many of these techniques (Iravani and Melville, 1981; Hermens and Merkus, 1987; Schipper et al., 1991). For example, studies of radioactive or radio-opaque particle transport in humans or animals and the saccharin test in humans are commonly used as methods for measuring nasal mucociliary transport time (NMTT). Unfortunately, these methods are frequently too costly for routine testing, are difficult to use in humans when studying non-approved agents, or cannot be used in whole animal models. The measurement of ciliary beat frequency (CBF) is another method used for studying the mucociliary activity in vivo in humans and animals and in vitro using respiratory epithelial cells or tissues, excised frog palates, or embryonic chick tracheal tissues. These techniques, while quantitatively quite accurate and reproducible, require specialized equipment for data acquisition and analysis. The use of excised tissues also brings into question the validity of any prediction regarding the effects on ciliary motility in vivo based on the changes which occurred in vitro. In addition, excised tissues only remain viable for a finite period of time, thus restricting these techniques to the evaluation of acute drug induced effects. Good correlations between the mucociliary transport time in human volunteers and ciliary beat frequency of chick embryo tracheas have been reported (Duchateau et al., 1985); it was observed that the greater the increase in nasal clearance time in humans, the greater the decrease in the ciliary beat frequency in the excised animal tissues. While the observed correlation provides some initial evidence validating the in vitro model, there are no methods currently available to predict the duration of effect of ciliostasis or hypermotility in vivo or to assure that the reversibility of an agent's effect observed in vitro reflects its in vivo behavior.

Several drug agents whose effects on ciliary motility have been previously studied in vitro were selected for use in the investigation of their long term effects on in vivo nasal clearance. A simple method was developed using non-surgically modified rats to measure the clearance of non-absorbable particles, FluoSpheres® – 5.0 μm sulfated latex particles, from the nasal cavity. In a manner similar to other in vivo testing procedures, this method measures clearance by collecting the marker as it enters the oral cavity following its transit through the nasal cavity. Since there are no surgical interventions involved, changes in clearance patterns can be investigated repeatedly over an extended time interval, thus enabling both the measurement of the duration of ciliostasis and the time course of recovery.

2. Materials and methods

Orange FluoSpheres® (2% w/v aqueous suspension) were purchased from Molecular Probes, Inc. (Eugene, OR). Upon dilution, the FluoSpheres® gave a linear fluorescence response up to a concentration of 0.02 mg/ml. Ketamine hydrochloride (10%) was obtained from Aveco Co., Inc. (Fort Dodge, IA). Lidocaine HCl (Pfaltz and

Bauer, Stamford, CT), pilocarpine nitrate (Mallinckrodt Chemical, St. Louis, MO), neomycin sulfate, tripelennamine hydrochloride and bacitracin (Sigma Chemical, St. Louis, MO) were used as received. The control solution for all experiments was normal saline (Baxter Healthcare Corp, Deerfield, IL). Drug solutions at identical concentrations to those previously studied in vitro: lidocaine HCl (2%), pilocarpine nitrate (0.15%), neomycin sulfate (0.5%), tripelennamine HCl (2%), and bacitracin (10000 U/ml), were made isotonic with NaCl (EM Science, Cherry Hill, NJ).

2.1. Animal studies

Male, Sprague-Dawley rats weighing from 250 to 300 g were used to measure the in vivo rate of mucociliary clearance. Rats were housed and experiments conducted in a temperature and humidity controlled environment. At least three animals were exposed to each drug compound. The rats were lightly sedated by subcutaneous injections of ketamine solution (0.25 ml every 30 min) throughout the experiment. 25 μl of the drug solution of interest were instilled into each of the animals' nostrils. The drug was allowed to be absorbed or cleared by natural mechanisms for the following 30 min, then an equal volume of FluoSpheres® suspension (25 μl) was instilled into each nostril for clearance measurement. The FluoSpheres® exiting the nasal cavity were collected by swabbing the oral cavity of the rat with moistened, cotton-tipped applicators every minute for the first 30 min following instillation and then every 5 min for the following 90 min. 4 ml of distilled water were used to extract the FluoSpheres® from the applicators, and the resulting solution fluorescence was measured using a fluorescence spectrophotometer (RF 540, Shimadzu Scientific Instruments, Columbia, MD). The mass of FluoSpheres® in the sample was then calculated from a standard curve determined in conjunction with the analysis of each group of clearance samples. The experimental procedures were repeated in the same animals at 24 and 48 h following drug exposure. On each of these days, normal saline was instilled into the nostrils instead of the drug solution. Identical normal saline instillation experiments were performed in each rat for at least 2 days prior to drug exposure to obtain control clearance rates for each group.

2.2. Data analysis and statistics

The clearance of FluoSpheres® from the nasal cavity was expressed in terms of the total mass (M_R) of FluoSpheres® recovered as a function of time (t). The value of the total mass recovered at the end of the 120 min collection interval (M_R^∞) was then used to transform the accumulated mass vs time relationship to % of total mass accumulated vs time. The resulting values obtained between 2 and 60 min were fitted to a monoexponential equation of the form $M_R = M_R^\infty - Ae^{-kt}$ where A is a constant. The clearance values obtained during the first 2 min were excluded to eliminate results attributable to volumetric clearance, which is not controlled by ciliary motility, from the data analysis. Nearly 100% of the clearance occurs within the first hour following FluoSpheres® instillation. Therefore, since little rate information can be obtained from the data obtained between 60 and 120 min, these data were not included in the fitting procedure. The time for 90% of the total FluoSpheres® mass to clear from the nasal cavity (t_{90}) was determined from the transformed data. Areas under the resulting exponential curves (AUCs) were calculated using the trapezoidal rule. The mean clearance rates (k), t_{90} values and AUCs for each animal group tested were compared to the observed mean control values for that group using the Student's t-test ($p < 0.05$).

2.3. Tissue morphology

Fresh samples of excised ovine nasal mucosa were immersed in a solution of each drug at the concentrations used in the previous in vivo studies. Each tissue sample remained in the drug solution for periods of either 30 or 120 min. The tissues were removed from the drug solution and placed into a 2.5% glutaraldehyde solution and allowed to fix overnight. The tissues were removed, dehydrated in a graded series of ethanol

solutions, critical point dried, sputter coated with silver, and examined using a Hitachi S4000 scanning electron microscope.

3. Results and discussion

Previous investigators have studied the effects of 2% lidocaine hydrochloride and 2% tripelennamine hydrochloride (Van de Donk et al., 1982a), bacitracin (10 000 U/ml) and 0.5% neomycin sulfate (Van de Donk et al., 1982b), and 0.15% pilocarpine nitrate (Iravani and Melville, 1975) on ciliary motility in vitro. Results using these compounds in the in vivo clearance studies are shown in Fig. 1 and summarized in Tables 1–3. From these data it is apparent that clearance from the nasal cavity can frequently be well described by a simple monoexponential function. While this form of equation does not fit each individual data set as well as a multiexponential or polynomial equation might, the monoexponential treatment simplifies data interpretation and allows direct comparisons between the clearance rates from different experiments to be made. Since the bulk of the clearance of FluoSpheres® in control animals generally occurs within the first 30 min following instillation, the most useful parameter for comparison was found to be the initial clearance rate (k). Obviously, for the cases that do not demonstrate exponential clearance behavior, the AUC or t_{90} values will more accurately describe changes in clearance patterns than will the rate constants. The AUC values alone retain little information regarding the clearance patterns observed, however, thus limiting their utility as a single parameter for comparative purposes. When coupled with the t_{90} parameter, an estimate of the rate of clearance, the AUC values become quite useful for the interpretation of drug effects on mucociliary clearance patterns.

The results from these in vivo studies show good correlation with those observed in previous in vitro studies and provide additional information regarding the duration of the drug effects on nasal clearance patterns. The most dramatic changes in clearance patterns are observed following the administration of lidocaine HCl (Fig. 1a) where a significant decrease in clearance rate (Table 1) and total particle clearance (AUC) (Table 2) is observed at both 24 and 48 h post-exposure. The corresponding t_{90} values are nearly 4-fold greater than those observed in the control studies. The in vitro results reported also indicated that lidocaine rapidly decreased the CBF, but the beating frequency slowly recovered after the lidocaine HCl solution was rinsed from the tissue section with Locke Ringer's solution (Van de Donk et al., 1982a). The effect of lidocaine on CBF has also been studied using other drug concentrations with a number of different experimental techniques. Corssen (1973) reported that 0.01% lidocaine caused no change, 0.1% slightly increased, and 5–20% significantly decreased ciliary activity in cultures of human bronchial or tracheal mucosae. Dudley and Cherry (1978) observed that 0.4–4% lidocaine solutions exposed to chick embryo tracheal rings for periods of at least 20 min significantly decreased ciliary activity. The tissues exposed to 0.4 and 1% lidocaine showed nearly complete recovery of ciliary activity following the incubation of the exposed tracheal rings with Hepes-Eagles basal medium (Hepes-BME) whereas the rings exposed to 2 or 5% lidocaine showed no signs of recovery. When 4% lidocaine solutions were instilled intratracheally first, followed by the excision and incubation of the tissues in Hepes-BME, no significant decreases in ciliary activity could be detected after 24 h. Rutland et al. (1982) also observed similar behavior in that cultures of human nasal respiratory epithelium showed significant decreases in CBF following exposure to 0.25–4% lidocaine. When the epithelium was exposed to 4% lidocaine immediately prior to harvest from the nasal cavity, however, no effect on CBF was observed. Fukuda et al. (1984) administered an i.v. lidocaine dose of 3 mg/kg to pigeons and measured changes in tracheal mucociliary transport time in situ. Significant decreases in transport rate were observed for up to 20 min following drug administration. The in vivo clearance results using 2% lidocaine HCl in rats showed only slight effects upon initial exposure, yet showed significant decreases in clearance at 24

and 48 h. From the varied in vivo and in vitro observations, it can be clearly seen that ciliary response to lidocaine HCl is dependent both on drug concentration and experimental methodology. Concentrations exceeding 0.25% consistently decreased ciliary motility in excised tissues or cell

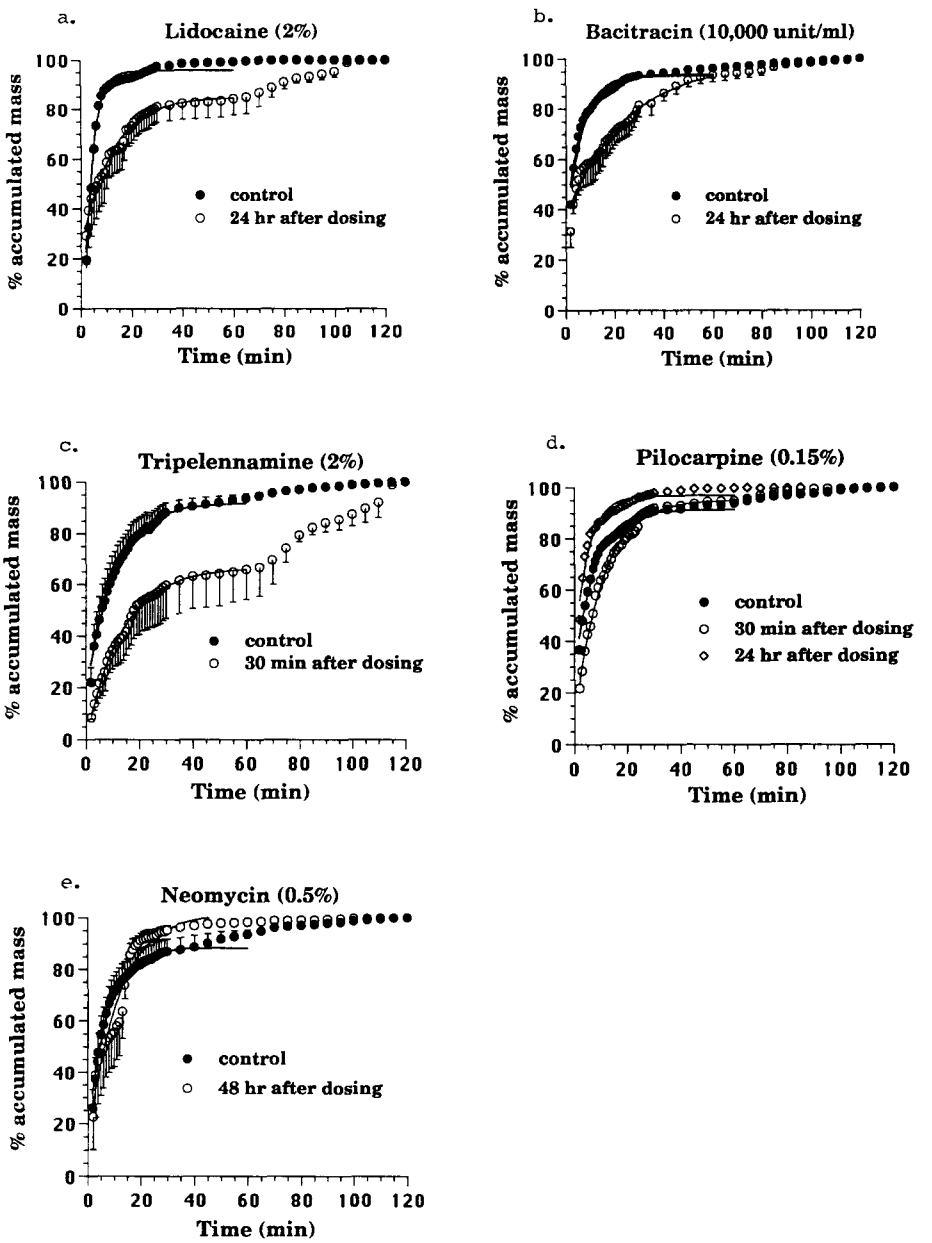

Fig. 1. FluoSpheres® recovery (±SE) following nasal administration of (a) 2% lidocaine HCl, (b) 10000 U/ml bacitracin, (c) 2% tripelennamine HCl, (d) 0.15% pilocarpine nitrate, and (e) 0.5% neomycin sulfate. Experiments were conducted for 2 days prior (control) and 30 min, 24 h, and 48 h post drug exposure. Each curve represents the best-fit monoexponential function for the 2–60 min data. Only the curves showing significant deviation from control are shown.

Table 1
Initial clearance rates

Drug	$k \pm$SE (min^{-1}) ($\times 10^{-2}$) (r value)			
	Control	30 min	24 h	48 h
Lidocaine	30.0±2.0 (0.91)	30.7±6.9 (0.90)	8.2±1.9 [a] (0.74)	6.7±0.9 [a] (0.68)
Bacitracin	15.0±1.3 (0.79)	12.3±2.3 (0.87)	4.1±0.9 [a] (0.84)	17.3±2.2 (0.78)
Tripelennamine	9.4±1.4 (0.69)	7.2±1.8 (0.73)	9.9±0.7 (0.96)	12.0±6.1 (0.42)
Pilocarpine	13.4±1.4 (0.83)	9.6±1.3 [a] (0.88)	19.2±1.9 [a] (0.91)	11.8±0.7 (0.97)
Neomycin	14.1±1.8 (0.77)	10.0±2.6 (0.85)	[b]	8.8±1.4 [a] (0.68)

[a] Statistically different: $p \leq 0.05$.
[b] Environmental controls not operating during experiment.

cultures. Concentrations up to 4% administered in situ, in comparison, showed little effect on mucociliary transport. Even though the results found in these in vivo clearance studies indicate that there are quantifiable differences in nasal clearance rates following exposure to 2% lidocaine, the fractional surface area exposed to the drug in these experiments was likely to be greater than that in tracheal instillations or in the studies of Rutland et al. (1982) where only one nostril of the human volunteer was exposed to an aerosolized lidocaine solution. The differences between these in vivo results and those previously reported for in vitro systems might also be due to the dilution of the applied concentration by secretions or clearance of the drug away from the site of application.

Table 3
Time for 90% Fluospheres® clearance (t_{90})

Drug	$t_{90} \pm$SE (min)			
	Control	30 min	24 h	48 h
Lidocaine	14±1.5	23±9.0	70±6.7 [a]	60±11.7 [a]
Bacitracin	22±6.7	15±3.3	50±5.7 [a]	15± 3.3
Tripelennamine	34±2.8	101±3.3 [a]	44±6.0	57±15
Pilocarpine	34±3.2	33±5.0	13±2.3	36± 7.0
Neomycin	35±7.3	23±6.7	[b]	20± 2.0

[a] Statistically different: $p \leq 0.05$.
[b] Environmental controls not operating during experiment.

Decreases in nasal clearance are apparent from the AUC at 24 h post-bacitracin administration (Fig. 1b). The clearance rate is also decreased, and the t_{90} is significantly increased (Tables 1–3). Unlike lidocaine HCl, however, all of the clearance measurements return to normal values within 48 h. In contrast, the in vitro results indicated that bacitracin irreversibly inhibits ciliary motility in an excised embryonic chick trachea model (Van de Donk et al., 1982b).

The clearance results following tripelennamine HCl exposure (Fig. 1c) demonstrate that clearance patterns do not always follow the simple monoexponential function characteristic of the control experiments. The clearance pattern at 30 min, in this case, resembles a sigmoidal function. Therefore, while statistically valid rate constants can still be obtained from the 0–60 min data, their value in assessing changes in total clearance is rather limited. In this case, the AUC and t_{90} parameters more accurately describe the changes in clearance caused by tripelennamine HCl. Both

Table 2
AUC values for FluoSpheres® recovery

Drug	AUC ± SE (% min)			
	Control	30 min	24 h	48 h
Lidocaine	11357± 47	11330 ± 211	9909 ± 323 [a]	9790 ± 269 [a]
Bacitracin	11126± 37	11375 ± 103	10325 ± 151 [a]	11385 ± 85
Tripelennamine	10625 ± 100	8058 ± 452 [a]	10464 ± 130	10217 ± 501
Pilocarpine	10866± 75	10718 ± 140	11527± 64 [a]	10898 ± 89
Neomycin	10661 ± 117	11247 ± 171	[b]	10976 ± 114

[a] Statistically different: $p \leq 0.05$.
[b] Environmental controls not operating during experiment.

parameters indicate a reduction in the efficiency of nasal clearance at 30 min following exposure but show a return to normal values within 24 h. In contrast, tripelennamine HCl, in vitro, irreversibly inhibited the CBF within 25 min at pH 5 and in less than 5 min at pH 6 and 7.4 (Van de Donk et al., 1982a).

Pilocarpine nitrate was observed to increase CBF in vitro using excised rat bronchi (Iravani and Melville, 1975). Following i.v. administration (160 mg/kg) pilocarpine was also shown to increase the rate of charcoal movement in a non-depleted frog palate system (Slaughter and Aiello, 1982). In a live chick model, the application of pilocarpine (0.6%) was observed to induce an initial hypersecretion of mucus and a subsequent increase in clearance, yet a normal clearance rate returned within 30 min. (Bang et al., 1966). Similar behavior is reflected in the in vivo clearance measurements at 24 h post-exposure (Fig. 1d) where the clearance rate and AUC indicate a significant increase in nasal clearance. The increase in clearance is not observed at 30 min, however. In fact, a slight decrease can be seen, yet it is of insufficient magnitude to be reflected in the AUC or t_{90} values.

For the case of neomycin sulfate, a compound that showed no effect on ciliary function in vitro (Van de Donk et al., 1982b), a slight decrease in clearance rate and a deviation from monoexponential behavior at 48 h are the only changes observed during the in vivo experiments.

It might be expected that the best correlation between in vitro and in vivo behavior would be found for the 30 min in vivo observation period due to the similarity in sampling time as compared to the in vitro experiments which measure CBF directly. Surprisingly, this was not the case; there is little correlation between the reported in vitro results and any changes in clearance patterns at 30 min. Instead, the best correlation exists at the 24 h observation period. One explanation for this delay in effect is that the drug concentration at the mucosal surface is likely to be significantly lower in the rat nasal cavity than on the excised tissue surfaces due to dilution in nasal secretions, normal clearance out of the nasal cavity, and drug absorption through the mucosal tissues into the systemic circulation. Therefore, the exposure time necessary to alter ciliary motility patterns is likely to be longer in vivo, occurring on the order of several hours rather than minutes post-exposure.

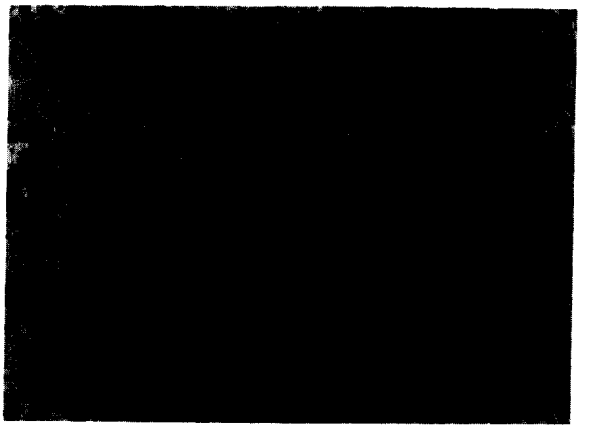

Fig. 2. Scanning electron micrograph of excised ovine nasal mucosa. Control tissue not exposed to drug solutions.

Very poor correlation of the observations regarding 'reversible' and 'irreversible' changes in ciliary motility was found between the in vivo and in vitro methods. For example, while the in vitro studies indicated that exposure to bacitracin irreversibly inhibits ciliary movement (Van de Donk et al., 1982b), the in vivo results show that even though there is a decrease in nasal clearance rate at 24 h, normal clearance patterns are resumed within 48 h. The best explanation for the irreversibility observed in the in vitro case comes from an examination of the surface of a sample of excised nasal mucosa exposed to bacitracin using scanning electron microscopy. Compared with normal tissue (Fig. 2), the tissues exposed for 30 min (Fig. 3) show significant morphologic changes in ciliary organization, and tissues exposed for 120 min (Fig. 4) are completely denuded of cilia. From the degree of morphologic change exhibited in these micrographs, it is not surprising that the reduction in ciliary beat frequency was not reversible in the excised tissue systems. Due to the dilution of drug concentration, the probable lack of complete mucosal surface coverage, and the ability to repair epithelial damage, the changes

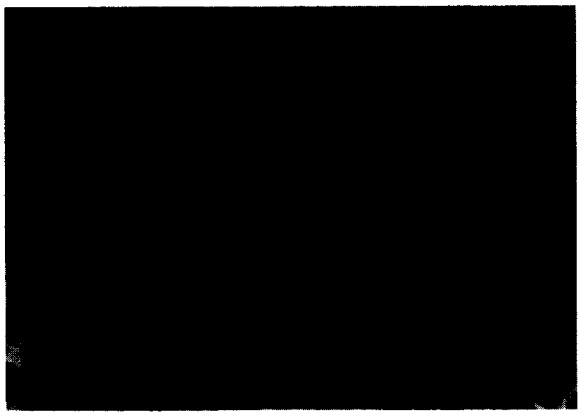

Fig. 3. Scanning electron micrograph of excised ovine nasal mucosa following exposure to 10000 U/ml bacitracin for 30 min. Alterations in ciliary organization can be observed.

which were observed in vivo were much milder in comparison and were reversible within 48 h.

Of the drugs tested, the only compound to show long-term effects in vivo was lidocaine HCl. Morphologic changes were also observed in the tissues exposed to lidocaine following both 30 (Fig. 5) and 120 min (Fig. 6), though they were not of the magnitude of those observed with bacitracin. While the extent of exposure to lidocaine in vivo is expected to be less than that of the excised tissue sample exposed for 30 min, the prolonged changes in clearance patterns indicate

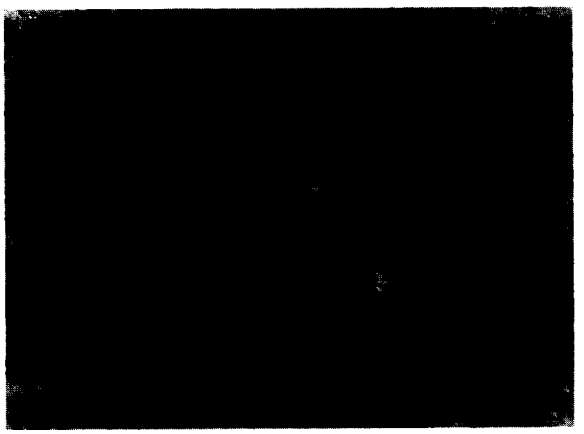

Fig. 4. Scanning electron micrograph of excised ovine nasal mucosa following exposure to 10000 U/ml bacitracin for 120 min. Tissue is completely denuded of cilia.

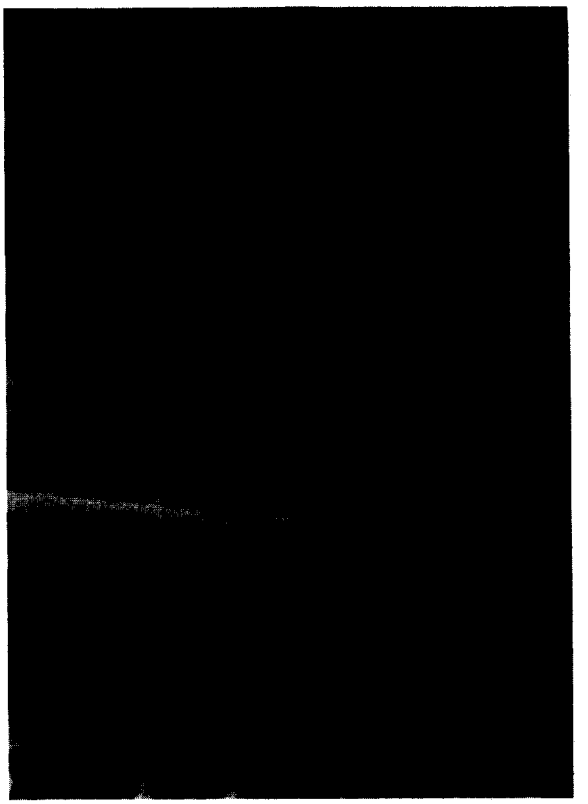

Fig. 5. Scanning electron micrograph of excised ovine nasal mucosa following exposure to 2% lidocaine for 30 min. Alterations in ciliary organization can be observed.

that there are mechanisms in addition to physical damage by which lidocaine induces ciliary stasis. Due to the lack of complete surface coverage and the dilution of drug solution which occurs in vivo, however, not all of the cilia present on the mucosal surface are likely to be affected by lidocaine. As a result, clearance from the nasal cavity still occurs due to the motility of the unaffected cilia, although at a somewhat slower rate than in control studies. Interestingly, lidocaine HCl is also currently used clinically as a topical anesthetic during nasal examinations or for minor surgical procedures. Its clinical acceptance and the lack of reported adverse effects which can be directly attributed to ciliary stasis provide supportive evidence that the changes observed in the in vivo clearance studies may be more useful for predicting clinical outcomes for acute use agents

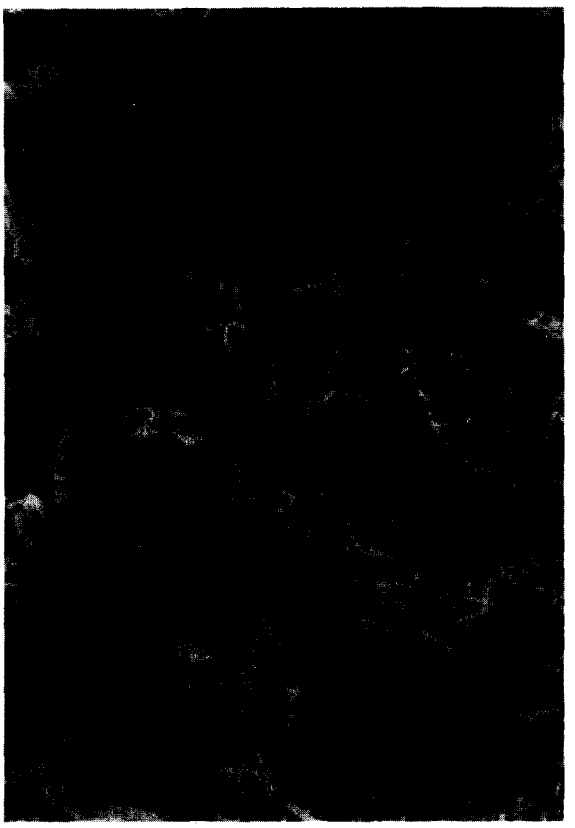

Fig. 6. Scanning electron micrograph of excised ovine nasal mucosa following exposure to 2% lidocaine for 120 min. Alterations in ciliary organization can be observed.

than are changes in ciliary motility observed using excised tissues.

4. Conclusions

The cilia present on the surface of the nasal epithelium serve a vital role in the mucosal defense system in the nasal cavity. Prolonged ciliary immotility can lead to the occurrence of nasal congestion, nasal infections, and other related pathologies. Therefore, it is important to be able to accurately estimate the degree of drug or formulation induced ciliary change in order to develop safe and effective nasal dosage forms. Drug effects on ciliary motility patterns can be easily and rapidly investigated with in vitro methods which measure the changes in ciliary beat frequency or mucociliary clearance rates using either excised or isolated tissues. When the results obtained using these in vitro systems are compared to rates of nasal clearance in whole animals, however, it can be seen that the in vitro results frequently overestimate the actual changes observed in vivo. Homeostatic control mechanisms (e.g., dilution of concentrated drug solutions by the nasal secretions, removal of drug from the nasal cavity via clearance or absorption, replacement of depleted endogenous substances (Ca^{2+}, ATP, glucose, etc.)) all take place within the intact mucosal tissues and cannot be easily replicated in excised tissues. This lack of homeostatic control, coupled with the finite period of time over which excised tissues remain viable, limits the usefulness of these models to accurately predict the degree and duration of changes in nasal clearance. By observing the rate of clearance of non-absorbed fluorescent particles from the nasal cavity, longer term changes in mucociliary clearance patterns due to acute or chronic drug application can be observed. Measuring the clearance of these non-absorbable particles at frequent intervals over a finite time interval allows both quantitative and qualitative estimates of the changes in mucociliary clearance patterns to be made. Frequently, the clearance of these particles out of the nasal cavity follows simple first order kinetics. Both the rate (k or t_{90}) and extent (AUC) of particle recovery in these cases can be used to quantitatively compare drug induced changes in clearance. When particle clearance does not follow first-order kinetics, first-order rate constants (k) cannot be used for comparisons, but t_{90} and AUC values can be used for limited quantitative comparisons and additional qualitative assessments of changes in clearance patterns. The in vivo clearance studies can also be performed repeatedly thus enabling the time course of recovery of normal clearance patterns to be followed. While excised tissue systems are well suited for mechanistic studies of drug/formulation effects on the cilia themselves (both morphologic and CBF effects), pairing these studies with whole animal clearance experiments can lead to a more reliable estimate of a nasal dosage form's clinical utility.

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