Toxicological Implications of Nasal Formulations

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Various nasal formulations have been tested for their suitability to deliver drugs through the nasal cavity. This route is especially of interest where the dose of drug is small and the drug may undergo an extensive first-pass metabolism and/or decomposition while passing through the gastrointestinal tract. Unfortunately, the nasal mucosa does not have same type of tolerability to all drugs and additives used in formulations. Some chemicals may damage the nasal epithelia or alter the mucociliary defensive mechanism of the nose. There also is a possibility that the drug can transport directly from nasal cavity to the brain via the olfactory route. Several methods have been developed to study the impact of drugs and excipients on the integrity of the nose. In some cases, the in vitro results did not correlate well with in vivo data, due to lack of reproducibility of the natural body environment, and some in vitro methods may not be sensitive enough and thus may complicate interpretation of the results. This review provides a toxicological evaluation of different drugs and additives used to optimize a nasal formulation. Certain chemicals are now routinely used as additives in nasal formulations. Although these compounds are most likely safe, if they are used over the long term, they may damage the epithelia of the nose. For multidose preparations, preservatives are often included in nasal delivery systems and may cause ciliotoxic effects. Both physicochemical parameters of drugs as well as formulation materials should be considered in evaluating the overall effect of a drug product on the nose. Therefore, any prior knowledge of the effect of drugs and additives on the nasal epithelia ultimately will assist in the development of nasal products. Furthermore, as the sites of absorption in the nasal cavity are somewhat limited, evaluation of the long-term tolerability of a nasal formulation is of great importance.

The recent literature reports that administration of certain drugs intranasally for systemic effect has proven to be very effective. The nasal route is especially advantageous as an alternative means for the delivery of drugs that undergo extensive first-pass metabolism or are sensitive to gastrointestinal decomposition (Zia, Dondeti, and Needham 1993). Many small molecules, like dihyroergotamine, metaclopramide, butarphanol tartrate, subistorphanol succinate, and larger molecules such as vitamin B_{12}, vasopressin, calcitonin, and even insulin have been successfully delivered intranasally. Although this route has a significantly higher potential impact on the bioavailability of drugs in comparison to other routes, several other factors may influence its viability. The mechanism of absorption of drug through the nasal cavity has not been fully elucidated.

A variety of drugs with different physicochemical factors are absorbed by the nasal mucosa (Hussain et al. 1980; Su, Campanale, and Gries 1984). It seems that neither hydrophobicity nor hydrophilicity is the sole determining factor for nasal absorption. The anatomy of the nasal mucosal barrier suggests that several separate compartments may contribute to the permeability of the transnasal passage of drugs. The existence of an aqueous boundary layer also may influence the transnasal absorption of both lipophilic and hydrophilic drugs (Krishnamoorthy and Mitra 1998; Roche 1977). Therefore, any change in the complex architecture within the nasal passage due to the nasal formulation may ultimately affect the bioavailability of drugs.

The nasal epithelium is covered by many hair-like cilia that beat in a coordinated manner within the periciliary fluid beneath a layer of viscoelastic mucus. This movement within the nose results in mucociliary clearance. After nasal inhalation, mucociliary clearance contributes to the body's primary nonspecific defense mechanism by entrapping such potentially hazardous materials as dust and microorganisms, allergens, carcinogens, and cellular debris within the mucus blanket. The entrapped materials are then propelled by the claw-like tips of the underlying cilia toward the pharynx and either swallowed or expectorated (Proctor 1977). Nasal medication should not influence this self-cleaning capacity of the nose. It has been shown that the "immotile cilia syndrome" leads to recurrent infections of the airways (Afzelius 1979; Duchateau et al. 1985) which is linked to the increased occurrence of bronchiestasis, bronchial infection, and chronic rhinitis.

Many drugs and additives have demonstrated inhibition of nasal ciliary movement. For instance, ciliostatic agents such as mercuric preservatives, antihistamines, dihydroxy bile salts, local anesthetics, and active agents like propranolol, atropine, and salmeterol reduce the ciliary beat frequency (Wanner, Salathe, and O'Riordan 1996; Kanthakumar et al. 1994; Hermens and

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Merkus 1987; Rutland, Griffen, and Cole 1982). Therefore, it is important to investigate the occurrence of a ciliostatic effect and if it is reversible after withdrawal of drug or excipient exposure. Any drug or additive used in nasal delivery should be devoid of serious ciliotoxicity since the overall feasibility of a nasal drug formulation may depend largely on the effects of the ciliated epithelial tissue. Although the nose is exposed continuously to airborne environmental chemicals as well as those substances present in the general circulation, this paper only reviews the effects of drugs and additives that have been recently evaluated in nasal drug delivery systems.

NOSE

An extensive description of the nose can be obtained from many of the available human anatomy and physiology books. The main features relevant to nasal delivery follow. The nostrils are a pair of nasal cavities divided by a nasal septum; their total volume is approximately 15 cc^3, with a total surface area of 150 cm^2. These nasal cavities are covered by a mucosa with a thickness of 2–4 mm, whose function in humans is 20% olfactory and 80% respiratory. The nasal epithelium has a relatively high permeability, and only two cell layers separate the nasal lumen from the dense blood vessel network in the lamina propria (Pontiroli, Calderara, and Pozza 1989). The human nasal cavity is lined with three types of epithelia: squamous, respiratory, and olfactory (Figure 1). The mucosa in the anterior part of the nose is squamous and without cilia. Within the anterior nostrils, a transitional epithelium is found that precedes the respiratory epithelium. The olfactory epithelium is present in the posterior part of the nasal cavity (Emmeline et al. 1995). The epithelium contains ciliary cells that produce a unidirectional flow of mucus toward the pharynx. A drug deposited posteriorly in the nose is cleared more rapidly from the nasal cavity than a drug deposited anteriorly, because clearance is slower at the anterior part of the nose than in the more ciliated posterior (Kublik and Vidgren 1998).

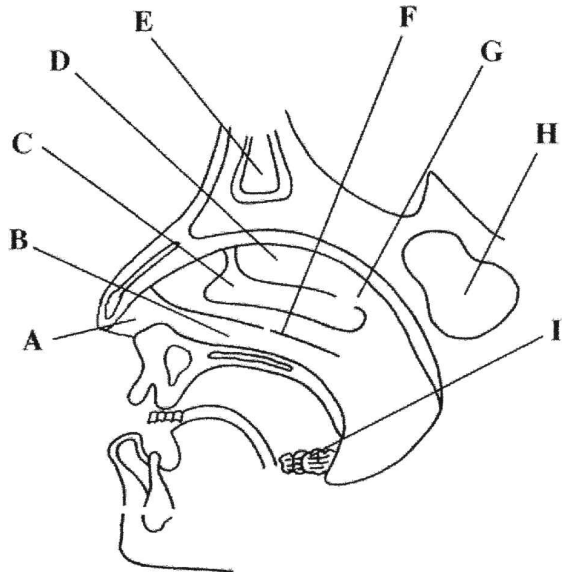

FIG. 1. Lateral wall of the nasal cavity. (A) Squamous epithelium, (B) inferior turbinate, (C) middle turbinate, (D) superior turbinate, (E) frontal sinus, (F) respiratory epithelium, (G) olfactory epithelium, (H) sphenoidal sinus, and

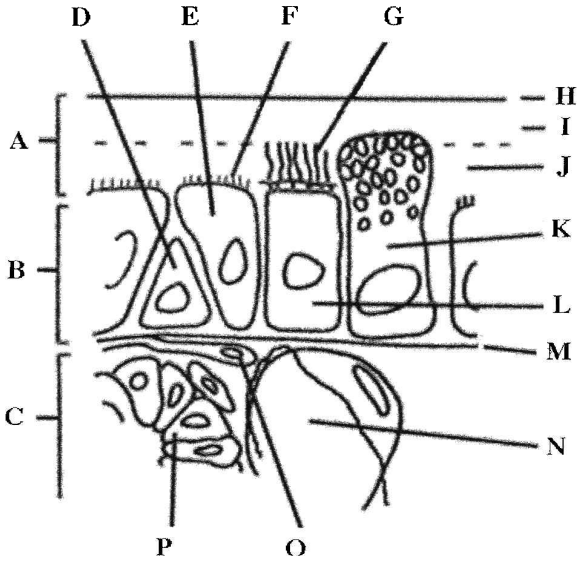

FIG. 2. Respiratory epithelium. (A) Mucus, (B) epithelium, (C) lamina propria/submucosa, (D) basal cell, (E) nonciliated cell, (F) microvilli, (G) cilia, (H) osmiophyllic membrane, (I) epiphase, (J) hypophase, (K) goblet cell, (L) ciliated cell, (M) basement membrane, (N) blood vessel, (O) nerve, and (P) gland.

The respiratory epithelium is the major lining of the human nasal cavity (Figure 2) and probably is the primary site for systemic absorption of nasally administered drugs (Monteiro-Rivera 1984). This epithelium is composed of ciliated and nonciliated columnar cells, goblet cells, and basal cells. The columnar and goblet cells are found on the apical side of the cell layer adjacent to the lumen of the nasal cavity. Basal cells are found adjacent to the basal lamina, on the basolateral side of the epithelium. The lamina propria is located beneath the basal lamina and contains many blood vessels, nerves, and glands.

METHODOLOGY TO EVALUATE NASAL FORMULATIONS

Most of the toxicological studies, both regulatory and academic, involve the use of whole animals. Today, we are influenced by the concept of the three Rs of humane animal use in research: replacement (utilization of models that do not involve live animals), reduction (use of fewer animals), and refinement (minimization of animal suffering). Thus, in vitro systems have widespread use for drug development studies (Reed 1997). For nasal delivery, several in vitro and in vivo models have been de-

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the integrity of the nose. These techniques include mucocilliary clearance, morphology of nasal mucosa, biochemical alteration or enzyme release, and changes in blood flow.

It also has been reported that there is a direct connection of nasal mucosa with the cerebrospinal and the central nervous system (Sakane et al. 1997). There is evidence of a phenomena in which cerebrospinal fluid leaks through the nasal mucosa to the nasal cavity without any underlying causes, when the intracranial pressure is elevated. Furthermore, an infectious organism has been shown to reach the olfactory nerve through the nasal mucosa (Tolley and Schwartz 1991). Thus, it is important to evaluate the potential impact of a drug formulation on the central nervous system when administering a drug intranasally.

Mucociliary Clearance

Several pathological conditions exist in which mucociliary clearance does not function properly. Primary ciliary dyskinesia syndrome, a group of congenital disorders also known as Kartagener's syndrome, is characterized by functional abnormalities of the cilia and subsequent impairment of the normal ciliary motility patterns in the respiratory and genitourinary tracts. The dysfunctional cilia within the respiratory tract are linked to increased occurrence of bronchiostatis, bronchial infection, and chronic rhinitis. If the application of drugs or formulation additives to the nasal mucosa results in similar patterns of dysfunction, it is likely that similar clinical pathologies may occur in chronic users of these medications (Donovan and Zhou 1995). Patients with cystic fibrosis also have impaired mucociliary clearance system, although their cilia are normal and function well (Middleton, Geddes, and Alto 1993). The mucus of cystic fibrosis patients has reduced water content, and the transport of this mucus has been observed to be delayed in vitro (Liote et al. 1989).

In case of viral and bacterial infections, the mucociliary clearance system is compromised, most likely due to a loss of cilia but possibly also to a change in the rheological properties of the mucus (Lindberg 1994). Hospitalized patients in intensive care units often have impaired mucociliary transport, which is associated with the development of pneumonia and retention of secretion (Konrad et al. 1994). In diabetes mellitus patients, who are susceptible to nasal infectious diseases, nasal mucociliary clearance time was found to be significantly larger than in a group of nondiabetic controls (Sachdeva et al. 1993).

To study mucociliary clearance, it is important to understand the pathology of the cilia. Cilia are motile hair-like appendages extending from the surface of epithelial cells. They beat in synchronized fashion in a highly complicated manner. The ciliary beat frequency (CBF) is regulated by several factors: temperature, intracellular calcium ion, cAMP levels, and by extracellular ATP. The CBF of human nasal cells in vitro increases with increasing temperature, between 5° and 20°C. Between 20° and 45°C, the frequency stabilizes at approximately 8–11 Hz and about 14 Hz between 32° and 37°C (Clary-Meinsez et al. 1992). The temperature dependency of cilia is mostly regulated by its axonemal enzymatic components, while the ciliary membrane has little effect. Extracellular ATP can increase the intracellular calcium level in cell cultures, resulting in an increased CBF. The ciliary beat frequency is also increased by increasing the levels of intracellular cAMP and cGMP (Lansley, Sanderson, and Dirksen 1992; Green et al. 1995).

As indicated, the function of mucociliary clearance is to protect the nose and the lower airways from damage by inhaled noxious substances; therefore, impairment of this system is potentially harmful. The efficiency of the mucociliary clearance system depends on the physiological control of CBF and on the rheological properties of the mucus blanket. Normal mucociliary transit time in humans is from 12 to 15 min. Transit times of more than 30 min are abnormal and are indicative of impaired mucociliary clearance. Thus, average rate of nasal clearance is about 8 mm/min, ranging from less than 1 to more than 20 mm/min (Andersen and Bende 1984).

Mechanical Devices to Evaluate Mucociliary Clearance

Many devices routinely measure CBF both in vivo and in vitro. High-speed cinematography estimates the frequency of ciliary waves; a video camera records the scene at high speed and afterward projects it at low speed for analysis. The time required for the camera to provoke a cessation of movement is estimated to assess ciliary activity (Gallay 1960; Sisson, Yonkers, and Waldman 1995; Gilain et al. 1993). With this technique, the cilia are illuminated with stroboscopic light flashing at variable cycles per second, which when equal to the frequency of the ciliary movement are perceived to the stationary (Andersen 1971). A photo multiplier transforms the light variations that result from mucociliary waves into voltage variations. After suitable amplification, the frequency is assessed (Dalhamn and Rylander 1962). In a second technique, the cilia from the rabbit oviduct are illuminated with a laser beam. The spectrum of the scattered light, when analyzed, provides information about the frequency of the ciliary movement (Lee and Verdugo 1976, 1977). A photoelectric registration device measures tracheal CBF. Light transmitted through the cilia is detected by a phototransistor mounted in a microscope, which measures the frequency instantaneously, displaying the waveform on an oscilloscope connected to a transient recorder (van de Donk, Zuidema, and Merkus 1980a). Photoelectronic detection is probably the most convenient means of quantifying CBF. It gives a complex spectrum of frequencies, but fast Fourier transform analysis of the analog signal gives a power spectrum of the fluctuating frequency. A disadvantage of using transmitted light is that only the beat frequency of the cilia at the edges of a piece of tissue explant can be measured. However, in cell cultures, it is possible to transmit light through the cell monolayer (Eshel, Crossman, and Priel 1985).

Different In Vivo/In Vitro Methods to Measure Mucociliary Clearance

Usually CBF is measured at body temperature (37°C), while the physiological range of nasal mucosa temperatures lies

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between 31° and 35°C. However, between 32° and 40°C, the nasal ciliary beat frequency was found to be independent of temperature. Optimal ciliary beat frequency was observed between pH values of 7 and 10. pH values lower than 6 and higher or equal to 11 resulted in larger decreases in the ciliary beat frequency of chicken embryo trachea. The activity of the ciliary beat is best evaluated in an isotonic solution (van de Donk et al. 1980a). The human amputated interior turbinate model was used to investigate the effect of chitosan on mucociliary transport rate (Aspden et al. 1997; Mason et al. 1995). Human turbinate begin to deteriorate approximately 4 hr postamputation; thus, to ensure that turbinate mucus exhaustion would not influence the results, all experiments were completed within 3 hr. After 15 min of chitosan contact, graphite particles were sprinkled over the surface of the turbinates and their movement was recorded. Human turbinates are in limited supply, and the tissue is more fragile and sensitive to suboptimal conditions than the other methods. However, this human model was found to give reproducible results, which alludes to the possibility of identifying differences between the effects of various compounds on mucocilliary clearance rate and could lead to greater accuracy in predicting the effects of nasally applied substances in clinical situations.

Clearance time also has been assessed by a standard saccharin taste test (Outzen and Svane-Knudesen 1993). This test is noninvasive and involves administering a saccharin formulation to one nostril and recording the time to taste the sweetness of the saccharin. The advantages of the saccharin clearance test as an indicator of mucocilliary clearance rate include its simplicity and relative inexpensiveness, which make it a routinely used and popular procedure in rhinology clinics.

Techniques involving excised frog palates devices and embryonic chick tracheal tissues (Dalhamn 1955) also have been used to evaluate the ciliary movement. These methods are too expensive for routine testing and sometimes problematic to test on humans or animal models. However, these techniques are quantitatively quite accurate and reproducible but require specialized equipment for data acquisition and analysis. In addition, excised tissues remain viable for a finite period of time, thus restricting these techniques to the evaluation of acute drug-induced or short-term effects. There are several advantages of the ex vivo methods described above for assessing ciliotoxicity, including the opportunity for simulating therapeutic dosage regimens under conditions in which the animal's natural defense mechanisms including mucus production and mucociliary clearance remain uncompromised.

Donovan and Zhou (1995, 1996) developed a nonsurgically modified rat method to measure the clearance of nonabsorbable particles from the nasal cavity. Similar to other in vivo testing procedures (the saccharin test), this method measures clearance by collecting the marker as it enters the oral cavity following its transit through the nasal cavity. The clearance pattern was investigated by measuring various kinetic parameters. The clearance of these particles from 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 a defined elimination order, the rate constant cannot be used for comparisons, but t_{90} and AUC values may be used for limited quantitative comparisons and additional qualitative assessments of changes in clearance patterns. These in vivo clearance studies also can be performed repeatedly thus enabling the time course of recovery of normal clearance patterns to be followed. Table 1 summarizes various types of approaches and methods used to evaluate the nasal toxicity of different drugs and excipients.

Although cilliary motility has been observed by many in vivo and in vitro methods, little correlation exists between these methods. Mucocilliary clearance rates are governed by interactions away mucus, cilia, and the intervening periciliary fluid. In vitro methods usually evaluate the effects of substances on individual components of clearance rates (i.e., CBF) and provide a good screening method for identifying substances with potential deleterious effects on nasal mucosal structure and/or mucocilliary function. However, they cannot totally substitute for in vivo investigations. The effect of various substances on nasal mucocilliary clearance rates, CBF, and the nasal epithelium when applied in a clinical setting cannot be predicted from in vitro approaches because factors such as dilution by mucus and limited contact with the mucosa cannot be accurately reproduced.

Histological Studies

Toxicological models were developed to compare the relative effects of different formulations on the morphology of the nasal mucosa. Scanning electron microscopy, used routinely to characterize the normal ultrastructure of the nasal respiratory epithelium, has proved an excellent technique for evaluating both gross structural alteration and specific cellular changes induced by exposure to different chemicals (Ennis, Borden, and Lee 1990). The main limitation of this type of technique is model design, with exposure conditions in test models often more severe than what is encountered in a clinical situation. Thus, the recorded histological alteration may not represent those observed after single or chronic dosing in the clinic. Furthermore, these models give a comparative qualitative evaluation that necessitates the use of multiple individuals with histopathological expert to evaluate the results.

Release or Alteration of Nasal Chemicals

Release of marker compounds from the nasal cavity also may evaluate potential damage to the nasal epithelium (Emmeline et al. 1995). Histological studies have disclosed that acid phosphatase activity is present in the squamous and respiratory epithelium. This activity is highest in the sensory cells of the olfactory epithelium, whereas the mucus and cilia do not contain acid phosphatase activity. The release of acid phosphatase is therefore an indication of nasal epithelial damage, especially in

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TABLE 1
Methods of evaluating nasal formulations

Method	In vivo/in vitro	Specimens	References
Clearance			
Video camera	In vitro	Guinea pig	Gallay 1960
	In vitro	Human	Sisson et al. 1995
	In vitro	Human	Gilian et al. 1993
Photoelectric	In vitro	Rabbit	Dalhamn 1962
	In vitro	Chicken embryo	van de Donk et al. 1980a
Laser beam	In vitro	Rabbit oviduct	Lee and Vardugo 1977
Cell culture	In vitro	Frog	Eshel et al. 1985
Interior turbinate	In vitro	Human	Mason et al. 1995
Saccharine taste test	In vivo	Human	Outzen et al. 1993
Frog palate mode	In vitro	Chicken embryo	Dalhman 1955
	In vitro	Frog	Batts et al. 1989
	In vitro	Frog	Gizurarson et al. 1990
Others			
Kinetic parameters	In vivo	Rat	Donovan et al. 1995
Morphology	In vitro	Rat	Lee et al. 1995
Release of marker	In vivo	Rat	Emmeline et al. 1995
Electrical membrane resistance	In vitro	Rabbit	Hosoya et al. 1994
Vasomotion effect	In vivo	Rabbit	Bende et al. 1992
Direct access to CNS	In vivo	Rat	Chou and Donovan 1996
Direct access to CNS	In vivo	Rat	Sakane et al. 1997

the olfactory region. Cholesterol also may be released in the nasal fluid as a result of the interaction of absorption enhancers with the nasal epithelium (Emmeline et al. 1995). The extent of release of total protein and the enzymes, such as lactate dehydrogenase (LDH) and 5′-nucleotidase (5′-ND), correlate with the extent of damage sustained by the nasal mucosa.

Membrane-bound 5′-nucleotidase release into the nasal perfusate gives an indication of the level of membrane perturbation, while release of LDH, a cystolic enzyme indicates the amount of cell leaching or lysis. The total protein release data, although not very specific as to the type of damage, provide a general indication of the extent of irritation (Shao and Mitra 1992a). Unfortunately, the analytical methods used to determine the levels of release of these marker compounds often are not highly sensitive and thus may complicate interpretation of the results. In addition, many excipients used in nasal formulation may inhibit the enzyme activity. Therefore, although the damage occurs, the enzyme activities may not be detected.

Membrane Resistance Measurement

Recently, the measurement of the electrical membrane resistance across the nasal mucosa has been used as a criterion to evaluate the damage caused by enhancers (Hosoya et al. 1994). Hosoya evaluated electrical membrane resistance (Rm) after applying absorption enhancers using the Ussing chamber technique. Although Rm values were kept constant in the absence of an enhancer, the value decreases drastically after application of enhancer. The magnitude of Rm change correlated well with the morphological changes shown by scanning electron microscope. It was postulated that a significant decrease in Rm resulted when an enhancer opened tight junctions or made new pore routes. But the behavior of Rm change with variation in exposure time and concentration of enhancer was not well documented. In this study, nasal mucosa were stripped from the nasal septum and placed in standard Ringer's solution. These excised tissues remain viable for a finite period of time, thus restricting the evaluation to short-term effects.

Vascular Reactions

The high vascularity and large surface area of the nasal mucosa make it a suitable site for rapid absorption of drug through the nasal cavity. Blood flow and therefore drug absorption may often depend upon the vasodilation and vasoconstriction of the blood vessels. Various methods have been used for experiential evaluation of vascular reactions in the nasal mucosa. Oxymetazoline has been shown to affect the vascular permeability of the nasal mucosa as a result of vasoconstriction (decrease in blood flow) and/or change in the permeability characteristics (Bende et al. 1992). In humans, changes in the blood content of the nasal mucosa have been studied indirectly using techniques such as

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