Nasal Mucosa as an Absorption Barrier

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REVIEW

Nasal Mucosa as an Absorption Barrier

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Key words: Nasal mucosa; Nasal absorption barrier; Transnasal absorption; Mucociliary clearance; Nasal enzymes; Nasal mucus

Summary

Transnasal absorption of pharmaceutical drugs has been recognized as an interesting alternative to the more conventional routes of administration. Even though the nasal mucosa can be used for transnasal drug delivery, it still remains an absorption barrier with specific constituants ensuring a protective function against foreign material penetration. The nasal mucosal barrier is organized at three different levels: a physical barrier composed of the epithelial cells and mucus, a temporal barrier controlled by the mucociliary clearance, and an enzymatic barrier acting principally on peptides and proteins. The mechanisms of action of such barriers are reviewed as well as some factors affecting them, and several absorption enhancement possibilities are discussed.

1 Introduction

In recent years, the nose has been recognized as an important alternative route for the delivery of therapeutics. The nasal cavity as a site for the systemic absorption of drugs has indeed some advantages (1):

- relatively large surface area (epithelial cells covered with microvilli)
- porous endothelial basement membrane
- highly vascularized epithelial layer
- high total blood flow per cm^3
- avoiding the first pass metabolism
- easily accessible

However the principal function of the nose, apart from olfaction, remains to condition the air (humidifying and warming), filter, and eliminate the airborne particles through the nasal mucociliary clearance. Consequently, the nose appears as a protective system against foreign material and the drugs administered in the nasal cavity will encounter some difficulties to pass through three different barriers:

- a physical barrier: mucus and epithelium
- a temporal barrier: mucociliary clearance
- a chemical barrier: enzymatic activity (for peptide and protein drugs in particular)

To gain a better insight into the functioning of these barriers and their effects on the drug absorption, this work will examine how epithelial cells, mucus, mucociliary clearance and enzymes influence nasal permeation and how this permeation can be modified by external factors. Clearly, a good comprehension of the factors limiting drug permeation through the nasal mucosa is essential for ensuring an adequate bioavailability for pharmaceutical drugs administered by this promising route.

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2 Anatomy of the Nasal Passage

The nose is divided into two symmetrical nasal cavities by the median septum, each half opens on the face through the nostrils (2). This nasal passage leads to the nasopharynx, it has a depth of approximately 12–14 cm, a surface of about 120 cm^2 and includes different epithelial areas (Fig. 1) (3):

- The vestibular area is covered by a stratified, keratinized and squamous epithelium including sebaceous glands and hairs which filter airborne particles (4).
- The atrium represents an intermediate zone lined with transitional epithelium, squamous at the anterior part and with microvilli at the posterior part.
- Behind the nostrils, three turbinates (superior, median and inferior) are located on the lateral nasal walls (5). The medium and inferior turbinates are lined by a pseudostratified columnar epithelium with cilia and represent the respiratory area (6).
- The olfactory area lies above the middle turbinate between the nasal septum and the lateral wall of the nasal passage. This epithelium contains specialized olfactory cells (5).

Heating and humidification of inhaled air are facilitated by the abundant blood flow through the arteriovenous anastomoses in the turbinates. Anterior serous glands, goblet cells and transudation from epithelial cells produce an important fluid supply (7). The area acting as a barrier for transnasal administration is the respiratory epithelium, a region usually reached by nasal drug formulations.

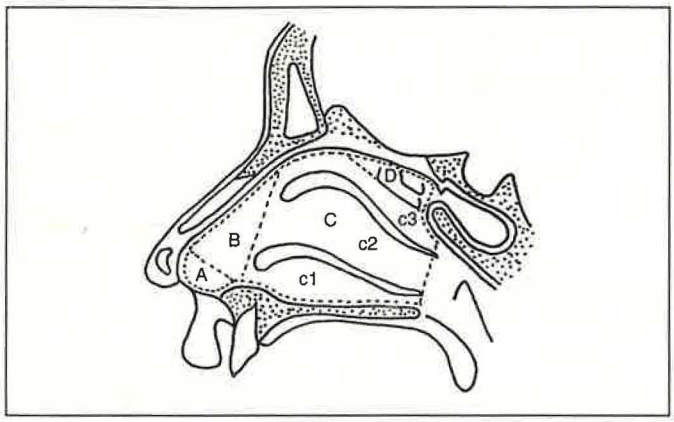

Fig. 1 Anatomy of the nasal cavity, modified from Crampette (119)
A = vestibule area, B = atrium, C = respiratory area, c1 = inferior turbinate, c2 = median turbinate, c3 = superior turbinate, D = olfactory area

3 Nasal Mucosal Barrier

3.1 Nasal respiratory mucosa

The respiratory mucosa lining the posterior two thirds of the nasal cavity is covered by a mucus layer and supported by a basement membrane. The respiratory epithelium consists of 4 types of cells (6) (Fig. 2):

- ciliated cells
- non ciliated cells
- goblet cells
- basal cells

In the area of the lower turbinate, approximately 20% of the total number of cells are ciliated. They have numerous microvilli (about 300) on their apical surface (8). Cilia are hair-like protrusions on the apical surface of the cell, which range in length from 5 to 10 μm and in width from 0.1 to 0.3 μm (9). The main function of the ciliated cells is to propel the mucus towards the pharynx by coordinated, wavelike movements of the cilia (8). Approximately 70% of the nasal respiratory epithelium on the inferior turbinate are not ciliated, but have numerous microvilli (about 400) on their apical surface. Their diameter is of about 0.1 μm and their length of up to 2 μm. These cells have a high metabolic activity and play an important role in fluid transport. They are characterized by the presence of extensive folding of the basolateral membrane, like high fluid transport

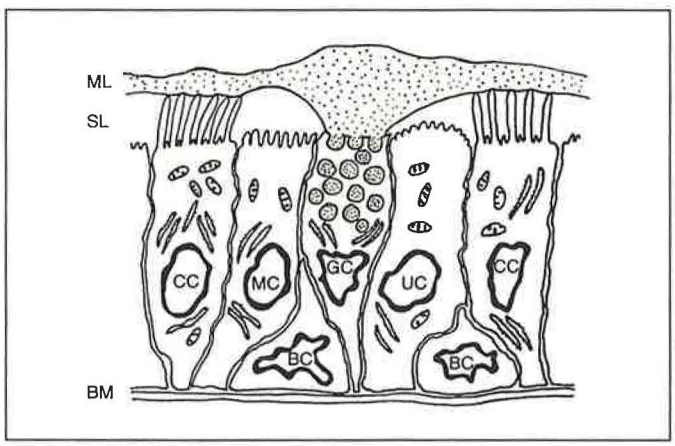

Fig. 2 Nasal epithelium cells
ML = mucus layer, SL = sol layer, CC = ciliated cell, MC = brush cell (cell with microvilli), BC = basal cell, GC = goblet cell, UC = undifferentiated cell, BM = basement membrane

cells, allowing facilitated fluid transport in and out the cells (8). Goblet cells represent almost 10% of the mucosal cells in the turbinates and contain numerous secretory granules. Their shape is produced by the distention due to the secretory granules in which the dehydrated mucus glycoprotein is stored (10). These granules produce secretions (complex carbohydrates) which form the mucus layer, together with submucosal glandular secretions (8). Basal cells are precursors of columnar cells (ciliated and non ciliated) and of goblet cells, via intermediate cells. They are poorly differentiated and never reach the epithelial surface (6). The epithelial cells are held together at their apical surface by tight junctions and act as a physical barrier against the permeation of foreign material.

The respiratory mucosa contains neurosecretory cells near the basement membrane. The connective tissue in the nasal mucosa is separated from the epithelium by the basement membrane and is of a loose type. Submucosal glands penetrate in the connective tissue and consist of both serous and mucous secretory cells.

Nasal capillaries characterized by the fenestrae of endothelium are apposed to the basement membrane (11). They are formed of large capillary loops which extend towards the respiratory epithelium (12). Between the venules and the capillaries numerous sinuses or venous lakes with erectile tissue are situated (13). The nasal vascular bed which lies under the epithelium is highly permeable and designed for a rapid passage of fluid and dissolved substances from the blood vessels to the tissues and vice versa (11, 14).

Despite the fact that the nasal epithelium forms a physical barrier to foreign material, some substances were found to pass through the nasal mucosa by different mechanisms: the nasal mucosa offers some measure of permeability.

3.2 Permeability of the nasal barrier

The nasal mucosa has a relatively high apparent permeability to both hydrophilic and lipophilic compounds (15). Several studies indicate that the nasal membrane may be characterized as consisting of a lipoidal pathway and of an aqueous pore pathway (16–19), except for Gibson et al. (20) who concluded from the lack of mannitol absorption that aqueous pores were not present in the nasal mucosa.

Transnasal administered drugs have to pass through the epithelial cell layer to reach their site of pharmacological action via the blood stream. Since nasal epithelium is composed of a layer of cells held together at the apical surface by tight junctions, drug passage through this barrier can theoretically occur (21) (Fig. 3):

- either via the transcellular route
- or via the paracellular route

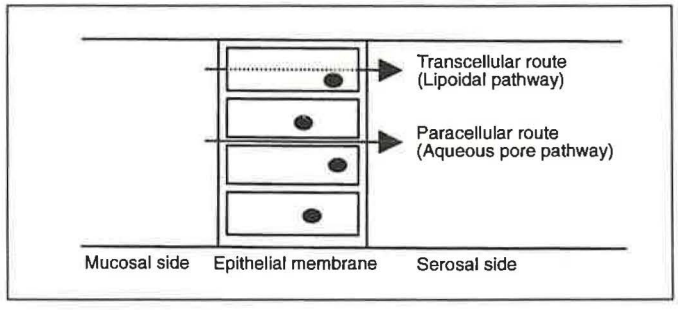

Fig. 3 Passage through the nasal epithelium

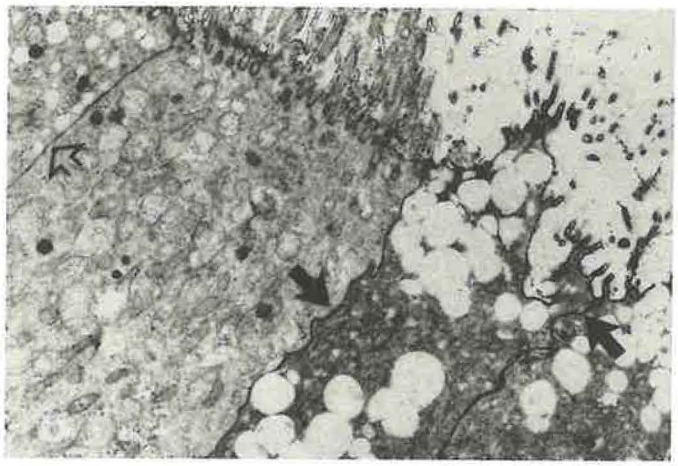

Fig. 4 Nasal polyp from patient with non-atopic chronic sinusitis
Intercellular spaces between a ciliated cell and a goblet cell or between goblet cells are filled with reaction product (full arrow), but little tracer is found in the intercellular space between ciliated cells (phantom arrow) (x 5100) [from ref. (26)].

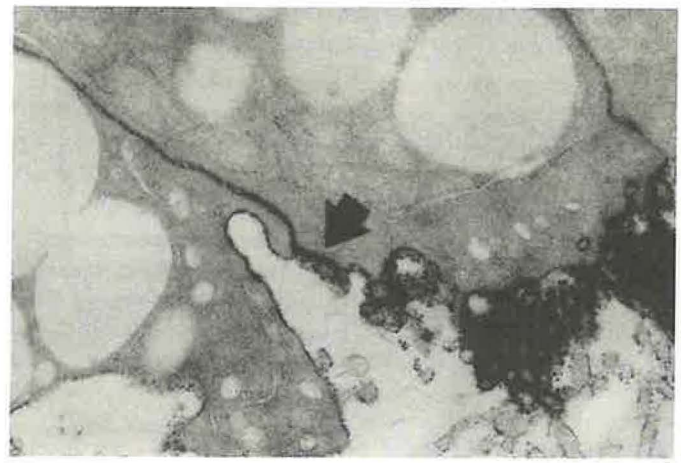

Fig. 5 Tight junctional region of nasal epithelium from a patient with allergic rhinitis
The tracer (Horse Radish Peroxydase) penetrates into the tight junction (x 15000) [from ref. (26)].

The **transcellular route** involves permeation across the apical cell membrane, the intracellular space and the basolateral membrane by passive transport (diffusion, pH partition hypothesis) or by active transport (facilitated and carrier-mediated diffusion, specific transcellular transport mechanism, endocytosis) (22, 23). This route is important for the absorption of lipophilic molecules or molecules capable of specific recognition of a membrane site (active transport). However, a proteolytic degradation can occur during the cytoplasmic passage of peptides and protein drugs (cf 5.) (24).

The **paracellular route** takes place between the epithelial cells by a passive process involving molecule diffusion across the intercellular junctional complex of the epithelium. Actually, tight junctions are dynamic structures which consist of plasma membranes brought into extremely close apposition, but not fused, so as to occlude the extracellular space. They can assemble or disassemble in response to various physiological stimuli. For instance, lowering extracellular Ca^{++} concentration and cytochalasin induce the opening of tight junctions (21, 22). The intercellular space between adjacent cells seems to vary with the regulation of fluid transfer across the epithelium (8). The plasma exudate may create intercellular spaces through which it can flow into the lumen (25).

Inagaki et al. (26) have observed that the connections between a ciliated cell and a goblet cell or between two goblet cells are relatively loose, and the connections between two ciliated cells are tight. This fact was illustrated by the presence of a tracer (Horse Radish Peroxidase: HRP) in the intercellular space (Fig. 4). Less tracer was found between ciliated cells than between goblet and ciliated cells.

As we can see, the permeability or the magnitude of the barrier is mainly controlled by the tight junctions (27), which form barriers between adjacent cells and act as rate-limiting portions to penetrants diffusing via the paracellular shunt pathway (28). The main pathway in a leaky epithelium for ionic penetrants is the paracellular one, this route being used by hydrophilic drugs like oligosaccharides and small peptides (22). Certain cells take up tracer proteins (HRP) by pinocytosis and transport them either to the lateral cell wall or to the basal portion of the cell where they are then released into the extracellular space.

It is interesting to note that the tight junctions become "leaky" in inflammatory and allergic conditions, allowing an intercellular passage of the substance (26) (Fig. 5).

Hayashi et al. (18) situate the pore size in nasal membrane at 3.9–8.4Å, and MacMartin et al. (17) have observed that molecules bigger than 1000 Da cannot pass through the nasal mucosa. Besides these purely physical properties, epithelia are known to possess certain charge characteristics and exhibit an electrochemical potential. Nasal epithelium possesses relatively high permeation characteristics and is slightly selective for the absorption of positively charged solutes (permselectivity) (27, 29, 30). The nasal mucosa is a relatively ineffective structural barrier because of its low membrane electrical resistance and its high permeability (28) which is largely confirmed by the absorption of peptides (17), PEG 5000 (31), PEG 4000 (23), sucrose (23, 31), mannitol (15), and DIT dextran (19) through the nasal membrane.

Several investigators have shown that the nasal absorption of compounds is influenced by many factors, including the hydrophilicity (16, 32, 33) as well as the molecular weight, the structure and the pka of the substance (17, 30, 34–37). In fact, the rate and extent of nasal mucosal absorption decrease as penetrant hydrophilicity increases (38).

According to Ungell et al. (39), a reversible opening of the paracellular route may be necessary to increase the absorption rate of peptides. In fact, the epithelial cells seem to regulate the junctional pathway by means of the cytoskeleton, and cytochalasin B was used in this study to increase the paracellular pathway of ileum and colon segments. This opening increases the flux of hydrophilic substances, such as vasopressin analogues, allowing them to be absorbed.

Some investigators have tried to enhance the absorption of drugs with substances like chelators (EDTA), cyclodextrins, synthetic surfactants, bile salts, fatty acids. These enhancers and their mechanisms of action have been recently thoroughly reviewed (21, 40–42) and the readers are invited to consult them for detailed explanations. Anyway, this is generally difficult to use absorption enhancers such as surfactants in nasal formulations because their toxicity is often too high and because they do generally not preserve the integrity of the nasal epithelium (43).

The nasal epithelium acts here as a barrier per se, but another factor which limits the transnasal drug absorption is the

function of the epithelium itself, a movement toward the pharynx: the nasal mucociliary clearance.

4 Mucociliary System

4.1 Beating of cilia

Mucociliary clearance is a protection system which is highly efficient for removing from the airways inhaled and deposited particles like allergens, toxic agents, bacteria and viruses. The efficiency of the nasal mucociliary clearance results from the interaction of epithelial cilia with the overlying mucus. It is dependant on several factors including (44):

- number and structure of cilia
- coordination and degree of ciliary activity
- mucus (volume, biochemical, physical, and rheological properties)

The ciliated cells transport mucus and trapped particles backwards to the pharynx with a flow rate of approximately 5–6 mm min^{-1}. The nasal cavity has a depth of 12–15 cm and thus the total contact time for any particle is 20–30 min (9).

Cilia are hair-like protrusions having nine pairs of microtubules and two central tubules. Each doublet contains an A and B subfibril with an inner and outer dynein arm (a complex protein with ATPase activity) located on the A subfibril with a radial spoke extending towards the central doublet (9) (Fig. 6). The motion of the cilia is dependant on the sliding of microtubules past one another. The energy for the ciliary movement is provided by ATP through dynein ATPase activity (45).

Cilia are surrounded by two distinct layers of mucus:

- a lower periciliary fluid (sol layer)
- an upper mucus layer (gel phase) with a higher viscosity than the sol layer (46)

Cilia beat in one plane with a fast effective stroke (the cilia dip into the gel layer and propel the mucus to the nasopharynx) and a slow recovery stroke (the cilia move backward through the sol layer). Cilia that propel mucus commonly beat at frequencies of between 10 and 20 Herz (9). The beating appears to pass from one region to another adjacent posterior one in a synchronized metachronous fashion (13) (Fig. 7). The nasal mucociliary clearance draws the particles towards the nasopharynx, preventing them from penetrating the nasal mucosa and allowing almost 20 min to an administered substance to be absorbed. This property of the nasal mucosa constitutes a temporal barrier to the transnasal drug absorption.

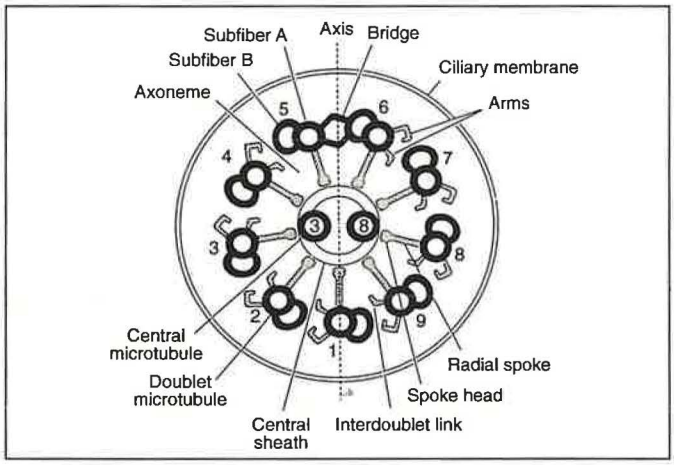

Fig. 6 Cilium cross-section [from ref. (120), cited by ref. (5)]

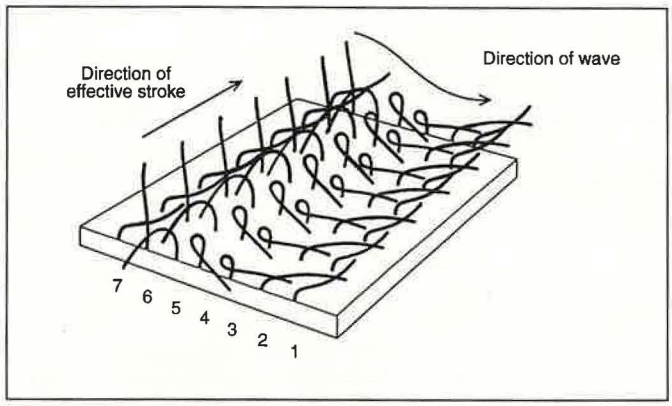

Fig. 7 Beating cilia [from ref. (120)]
Metachronal wave, usually perpendicular to the direction of the effective stroke
The cilia in row 1 are at the end of the planar effective stroke, those in rows 2, 3 and 4 are in successive stages of the curling return stroke, those in row 5 have ended the return stroke and are beginning the effective stroke, which is ended in row 7.

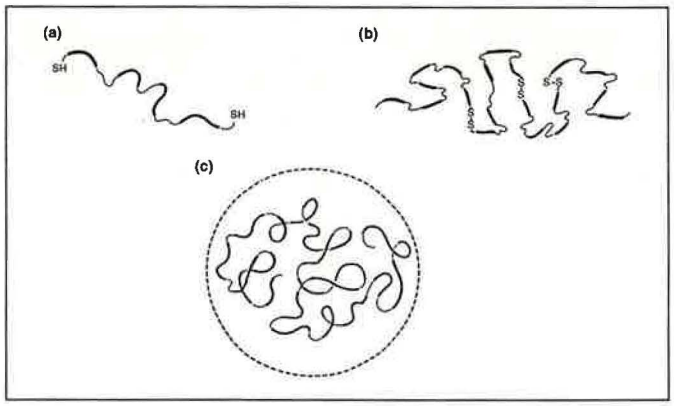

Fig. 8 Schematic illustration of the architecture of mucus glycoproteins [modified from (50)]
(a) Subunits constituted of oligosaccharides (~) and proteins (~)
(b) End-to-end association of subunits via disulphide bonds
(c) Random-coiled mucus glycoprotein

Continuous flow is maintained because of the viscoelastic nature of the mucus and the coherence of the mucus sheet, despite irregularities of the ciliary beat (47). The nasal secretions play consequently an important role in the nasal mucociliary clearance and it is interesting to examine them more in detail.

4.2 Nasal secretions and mucus

Nasal secretions originate from various sources like goblet cells, nasal glands, lacrymal glands and transudate from plasma. The submucosal glands contribute quantitatively much more than surface epithelial cells to the mucus layer surrounding the cilia. Approximately 1500–2000 ml of mucus is produced daily (5) and the nasal mucus turnover takes place in 10–15 min.

The nasal tissue fluids consist of three main components (1):

- water (95–97%)
- secretions (2–3%)
- electrolytes (1–2%)

Secretions from nasal mucus cells are composed of proteins such as proteolytic enzymes (aminopeptidases), secretory proteins (lactoferrin) and plasma proteins (antibodies), and glycoproteins (sialomucine, sulphomucine, fucomucine) (1, 7). The

sol phase contains water and soluble proteins and the gel phase is composed of water and of high molecular weight glycoproteins ($5x10^6$ daltons), called mucins (48). These mucus glycoproteins are stored in a dehydrated state and spontaneously rehydrate upon release to produce droplets which are drawn out into strands by the action of the beating cilia (10, 47). Mucines are the essential structural elements of mucus, responsible for the gel-like structure of mucus. This molecule consists of a polypeptide core and of rather short (2–18 residues long) sugar chains attached together through O-glycosidic linkages (48, 49). The mucin has a linear structure with subunits linked end-to-end via disulphide bonds or protein links (10, 50).

Glycoprotein chains are entangled, forming a network of long, fibrillar and randomly-coiled mucin, which consequently possess a three dimensional structure containing a large quantity of water (10, 51) (Fig. 8). It is interesting to mention that Girod et al. (52) support the hypothesis that the serous cells of the respiratory submucosal gland are able to synthesize, store and release phospholipids in the airway lumen.

The mucus gel layer, which occurs as a continuous blanket, spreads over the tips of the cilia; this property is called wettability. The mucus spreads as droplets or strings that may coalesce into larger rafts or sheets that are carried away by the cilia. While a cilium penetrates and pushes forward a section of mucus sheet, energy is stored elastically in the mucus, and the mucus recoils slowly, if allowed to, unless other cilia propel it further forward (53). As we can see, the rheological properties of the mucus are very important for a good mucociliary clearance (54). Pseudoplasticity, elastothixotropy, spinability and adhesiveness are all rheological characteristics of a respiratory mucus. The mucus is commonly described as a non-Newtonian viscoelastic gel, meaning that it has the ability to flow and deform (44). The gel is extremely sensitive to shear (55) and the transport rate is significantly correlated with the spinability (thread-forming ability) (56). The viscosity of mucus increases in parallel with molecular weight and intermolecular entanglement, and the elasticity increases with the frequency of intermolecular cross-links (48). The entanglement density, rather than the degree of covalent crosslinking, must regulate mucus rheology. The rheological properties of the mucus are consequently determined by the degree of hydration (whereas the number of entanglements per unit volume of gel is dependent upon hydration) (51).

King et al. (57) found that for a given total depth of serous and mucous layer, there exists a serous fluid layer thickness for which the mucus transport is maximum. The adhesiveness of respiratory mucus (mainly related to its surfactant properties) plays an important role in the capture of bacteria and aerocontaminants as well as the cilia mucus interaction.

The barrier properties of the mucus layer produced by intestinal goblet cells have been investigated by Karlsson et al. (58) and showed a significant effect on testosterone absorption, but the role of mucus in the absorption of drugs has not been yet established.

In the nasal mucosa, the thickness of the total mucus layer is low (about 4 μm) compared to the gut (100-fold greater), and the nasal mucus as an absorption barrier was not specifically investigated. Anyway, the drug has to diffuse through the mucus layer in order to reach the mucosal cells, and it could provoke a possible drug-mucus interaction.

The mucus barrier appears here as a physical barrier which can be affected by a change in its viscosity, provoking alterations of the mucociliary clearance.

4.3 Factors affecting the mucociliary clearance

Mucociliary clearance is dependent on a wide variety of factors including for instance the presence of dust and particulate matter in the air, allergy, nasal polyps, local infections, and

Table 1 Effects of some nasal diseases on the nasal mucociliary clearance

Disease	Effects on mucociliary clearance	Causes	References
Allergic rhinitis	↗	– alkaline nasal secretions – increasing of ciliary activity	(61)
Atrophic rhinitis	↗	– ↗ air pushing particles towards the nasopharynx	(61)
Nasal polyps	↘	– blockage of the nose – ↘ ciliary activity – ↘ mucus secretion	(62, 63)
Kartagener's syndrome	↘	– non motility of the cilia (absence of dynein arms)	(64)
Chronic sinusitis	↗ ↘	– ↗ ciliary activity in bathing in pus (↗ pH) – ↘ periciliary fluid – thetering of mucus	(61, 65) (66, 67)
Deviated septa or rhinoscleroma	↘	– obstruction – ↗ viscosity of mucus	(61) (68)
Cold	↗ (1st step) ↘ (2nd step)	– ↗ mucus flow – nasal congestion	(69)
Cystic fibrosis	↘	– abnormality of mucus	(70)

colds. The rate of propulsion of mucus appears to be relatively independent of the load (59) and an optimal ciliary beat frequency seems to occur at a pH of 7.0–9.0 (60).

Diseases of the nose produce changes in mucus secretion, pH and viscosity, ciliary motility, which contribute to the variation of the nasal mucosal barrier (1) (Table 1).

Several factors affect the mucociliary clearance by reducing the mucus transport (21):

- an increase in the depth of periciliary fluid (the effective stroke does not reach the mucus (47))
- an increase in the depth or viscosity of mucus (60)
- tethering of mucus to the mucus glands
- alterations in the ciliary cytoarchitecture

These factors have a negative effect on nasal mucociliary clearance, but could be seen as an advantage for enhancing the drug residence time and consequently promoting the drug absorption through the nasal mucosa.

A wide variety of ciliary structural changes have been noted in virtually every kind of chronic upper respiratory disease (39). Infections causing immunological stimulation rapidly induce the formation of goblet cells and the gel mucous layer seems to increase in thickness, thereby trapping more efficiently foreign material (8). However, it is interesting to note that according to Inagaki et al. (26), an increase in the number of goblet cells results in an enhancement of epithelial permeability due to the "loose" connections of the goblet cells between themselves.

Some other factors can influence the mucociliary clearance, like the use of contraceptive pills which can lead to symptoms similar to those of chronic hypertrophic non allergic rhinitis (71). The presence of an airway resistance determined by the submucous volume of the venous plexus: the nasal cycle (72), could have an important effect on the clearance rate. In fact, this cycle alternates with an unpredictable frequency of between 2 to 6 h and could provoke fluctuations of the mucus secretions with a similar time pattern.

It is obvious that nasal delivery of drugs is affected by rapid mucociliary clearance that sweeps foreign material towards the pharynx. The removal of labelled drug particles by mucociliary clearance from the initial site of deposition was clearly illustrated by gamma camera pictures (73). Several investigators have tried to enhance the transnasal drug absorption by prolonging the residence time of drugs, thereby diminishing the effect of the nasal mucociliary clearance by different bioadhesive formulations:

- microspheres (74–82)
- gels (80, 83–88)

Nevertheless, the balance of the nasal mucociliary function is very fragile, that is why these formulations have to respect its integrity and should not act by reducing the efficacy of this barrier.

The nasal mucociliary clearance appears as a temporal barrier controlled by both the cilia and the mucus. The movement drawing foreign material to the nasopharynx can be altered by a variety of factors and the residence time of the particle on the nasal mucosa can vary significantly. It is consequently very difficult to predict the capacity of the mucociliary clearance as a barrier against penetration.

5 Enzymatic Barrier

In addition to the permeation barrier, there also exists an enzymatic barrier to nasal drug delivery, which is created by metabolic enzymes of the nasal cavity. The nasal cavity was previously thought to possess little metabolizing capacity, but new evidence has demonstrated the presence of several proteolytic and secretory proteins (89, 4, 90). Chung et al. (91) have demonstrated that in addition to the metabolic activity within the mucosal tissues, the enzymes present in the nasal secretions are also highly active against peptide compounds. In fact, the enzymatic barrier of the nasal mucosa creates a pseudo-first-pass effect (92).

The nasal mucosa is known to contain both exopeptidases (aminopeptidases, diaminopeptidases, dipeptidases, etc...) and endopeptidases (serine proteinase, cystein proteinase, metalloproteinase, etc...) that cleave respectively the N- or C- terminal peptide bond and internal peptide bond of proteins and polypeptides (40, 93).

Aminopeptidases, well represented in the nasal cavity, are distributed throughout the cells to degrade peptides and proteins both during and after absorption into the cell. Aminopeptidase activity in the nasal mucosa has been found to be similar to that of the ileal mucosa in its subcellular distribution (89, 94). According to Lee et al. (40), almost half of the aminopeptidase activity in the nasal mucosa of the albino rabbit is membrane-bound, in comparison to the 80% in the ileal mucosa. Aminopeptidases N and A are plasma membrane-bound peptidases, and aminopeptidase B is a cytosolic enzyme (89).

According to Lee et al. (95), endopeptidases are involved in the degradation of substance P, insulin and proinsulin, whereas Gizurarson et al. (96) have reported that insulin was not significantly degraded by nasal enzymes. Some in vitro results indicate that aminopeptidases can be controlled by inhibitors of proteases (97) (Table 2).

Sarkar et al. (92) as well as Lee et al. (40) have reviewed the proteolytic enzyme inhibitors as absorption enhancers. Bestatin and puromycin are known to be potent aminopeptidase inhibitors but they alter the nasal mucosa and a rebond of aminopeptidase activity has been observed in the nasal mucosa after exposure to these inhibitors (101).

Both Hirai et al. (113) and Stratford et al. (89) have demonstrated the inhibitory effects of certain bile salts and derivates on aminopeptidases, but their toxicity is too high and they perturbe the membrane integrity. α-aminoboronic acid derivatives are excellent inhibitors of the degradation of peptides in the nasal mucosa by aminopeptidase (98) and have the advantage of efficacy at very low concentration and reversibility of effect (101).

It is interesting to note that peptidases in the nasal mucosa can be transciently inhibited via the coadministration of pharmacologically inactive peptidase substrates like the phosphinic acid dipeptide analogue used by Hussain (111) et al. to stabilize Leu-Enk (114).

Besides these proteolytic enzymes, the respiratory section of human nose contains a wide array of oxidative and non oxidative enzymes, which could play a crucial role in the bioactivation or detoxication in situ of inhaled xenobiotics (115, 116). The nasal mucosa, in particular the olfactory region is rich in cytochrome P-450 enzymes that metabolize inhaled pollutants into reactive metabolites which may induce nasal tumors (92). It has been reported that the metabolism of many compounds such as nasal decongestants, nicotine and cocaine could have a toxic effect in the nasal cavity (117).

Levels of human respiratory cytochrome P-450 are approximately 1/20 of those in the liver (118). The nasal cytochrome P-450, unlike hepatic cytochrome P-450, is relatively resistant to induction by xenobiotics, but is easily inhibited by common

Table 2 Several inhibitors of the nasal mucosa enzymes

Inhibitors	Enzymes involved	Substrates tested	Models	References
Aminoboronic acid derivatives		Leu-Enk	Rat	(98)
Boroleucine	Aminopeptidase	Thymopentin	Rat	(99)
		Leu-Enk	Rat	(100, 101)
		LH-RH	Rat	(102)
Amastatin	Aminopeptidase A	Growth Hormone	Rat	(103)
			Rabbit	(104)
		Leu-Enk		
Bestatin	Aminopeptidase B	Leu-Enk	Rat	(101)
Puromycin	Aminopeptidase B	Leu-Enk	Rat	(101)
Bacitracin	Aminopeptidase	LH-RH	Rat	(105)
		Buserelin	Rat	(105)
		ACTH	Rat	(106)
1,10 phenanthroline	Endopeptidase	–	–	(93)
p-hydroxymercuri-benzoate	Cysteine-proteinase	–	–	(93)
Thimerosal	Carboxypeptidase	Leu-Enk	Rabbit	(104)
EDTA	Enkephalinase	Leu-Enk	Rabbit	(104)
Na taurodihydrofusi-date (STDHF)		Insuline	Rat, rabbit	(107)
Na glycocholate	Proteases	Insuline	Rabbit	(108)
		ACTH	Rat	(106)
		Angiopeptin	Human, rabbit	(109)
		Leu-Enk	Rat	(110)
Phosphinic acid dipeptide analogue	Aminopeptidase	Leu-Enk	Rat	(111)
Polyoxyethylene-9-lauryl ether	Proteases	Insuline	Rabbit	(108)
Camostat mesilate	Aminopeptidase	Vasopressin	Rat	(112)

inhibitors of hepatic cytochromes P-450 (116). Despite its relatively low tissue concentration, nasal cytochrome P-450 is more active than the hepatic one in the metabolism of the substrates investigated (118). Metabolism by nasal cytochrome P-450 among other enzymes has been extensively reviewed by Dahl et al. (116). The nasal enzymatic degradation plays an important role in protein and peptide drug absorption and really acts as a non negligible chemical barrier to penetration.

6 Conclusion

The nasal mucosa represents a complex barrier to the drug absorption, which includes three different components: a physical, a temporal and a chemical one. The nasal barrier is necessary and has important functions, like olfaction, humidifying and warming of the air, elimination of foreign particles; therefore its integrity has to be maintained. This consideration is very important in regard to the absorption enhancers tested by some investigators, knowing that most of the actual enhancers could represent a "threat" in regard to the efficacy of the nasal mucosa. Actually, the permeation enhancers can act on different sites:

- the mucus, by diminishing its viscosity and promoting the drug diffusion through the mucus layer
- the epithelial cells, by opening the tight junctions and promoting the transcellular passage
- the mucociliary clearance, by slowing down the cilia beat and enhancing the residence time of the substance
- the enzymatic activity, by using enzyme inhibitors or analogues.

It is obvious that all these actions could weaken the nasal mucosa, allowing foreign material to penetrate in the body. That is the reason why the biggest difficulty remains to find effective absorption enhancers with a short lasting and a reversible action, in order to preserve the original functions of the nasal mucosa.

In conclusion, a good understanding of the mechanisms involved in these barriers is essential if we are to succeed to this fabulous challenge: permeate the nasal barrier in a reversible way, avoiding toxic effects on the nasal mucosa.

7 References

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