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Review Article

The biopharmaceutical aspects of nasal mucoadhesive drug delivery

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Abstract

Nasal drug administration has frequently been proposed as the most feasible alternative to parenteral injections. This is due to the high permeability of the nasal epithelium, allowing a higher molecular mass cut-off at approximately 1000 Da, and the rapid drug absorption rate with plasma drug profiles sometimes almost identical to those from intravenous injections. Despite the potential of nasal drug delivery, it has a number of limitations. In this review, the anatomy and physiology of the nasal cavity, as well as ciliary beating and mucociliary clearance as they relate to nasal drug absorption, are introduced. The rationale for nasal drug delivery and its limitations, some factors that influence nasal drug absorption, and the experimental models used in nasal drug delivery research are also reviewed. Nasal mucoadhesion as a promising method of nasal absorption enhancement is discussed, and factors that influence mucoadhesion, as well as safety of nasal mucoadhesive drug delivery systems are reviewed in detail.

Nasal drug administration is presently mostly used for local therapies within the nasal cavity. Anti-allergic drugs and nasal decongestants are the most common examples. However, nasal drug administration for systemic effects has been practised since ancient times. Nasally-administered psychotropic drugs by native Americans, the use of tobacco snuffs, and nasal administration of illicit drugs such as cocaine are all well known (Illum & Davis 1992). Nowadays, the nasal cavity is being actively explored for systemic administration of other therapeutic agents, particularly peptides and proteins (Illum 1992; Edman & Björk 1992), as well as for immunization purposes (Lemoine et al 1998). To better understand the basis for nasal drug absorption and factors that can influence it, a brief review of the anatomy and physiology of the nose is appropriate.

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Anatomy and physiology of the nose

Anatomy

The human skull is composed of two functional sections that protect the delicate structures within them. The neurocranium surrounds and protects the brain while the viscerocranium surrounds and protects the eyes, the mouth and the nasal cavity (Ridley et al 1992). The nasal cavity is divided into two symmetrical halves by the nasal (middle) septum and extends posteriorly to the nasopharynx. The most anterior part of the nasal cavity, the nasal vestibule, opens to the face through the nostril (Figure 1). The atrium is an intermediate region between the vestibule and the respiratory region. The respiratory region, the nasal conchae or turbinates, occupies the major part of the nasal cavity. It possesses lateral walls that divide it into three sections comprising the superior nasal turbinate at the top. Below this is

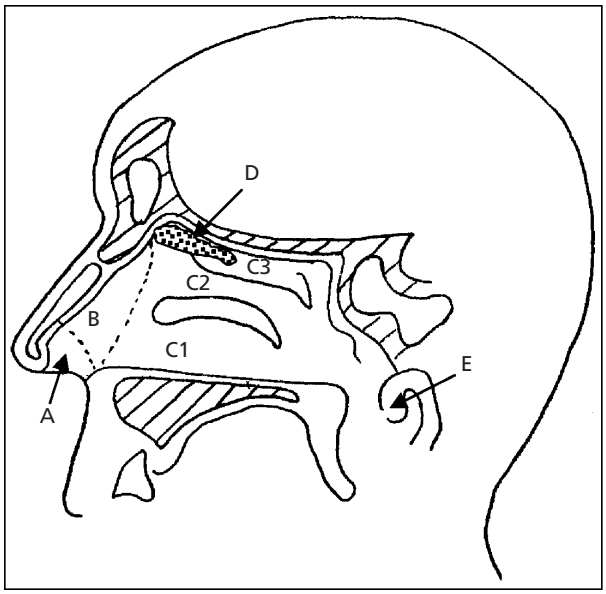

Figure 1 Saggital section of the nasal cavity showing the nasal vestibule (A), atrium (B), respiratory area: inferior turbinate (C1), middle turbinate (C2) and superior turbinate (C3), the olfactory region (D), and nasopharynx (E).

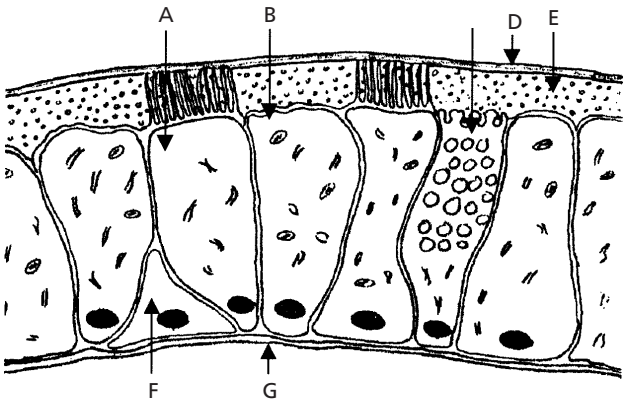

Figure 2 Cell types of the nasal epithelium showing ciliated cell (A), nonciliated cell (B), goblet cell (C), gel mucus layer (D), sol layer (E), basal cell (F) and basement membrane (G).

the middle nasal turbinate. The lowest chamber is the inferior turbinate. These folds provide the nasal cavity with a very high surface area compared with its small volume.

Morphology and physiology of the nose

The basic functions of the nose are heating and humidification of inspired air before it reaches the lungs, olfaction, resonance, filtration of particles, mucociliary clearance, and antimicrobial, antiviral and immunological activities (Druce 1986). The anatomy of the nose and functions of the epithelial cells at different regions of the nasal cavity are such that these functions are performed optimally. The olfactory region situated above the superior nasal turbinate (Figure 1) possesses specialized ciliated olfactory nerve cells for smell perception. The central axons of these nerve cells pass through the cribriform plate of the ethmoid and into the olfactory bulb (Ridley et al 1992). The total surface area of the olfactory epithelium is 200–400 mm^2 (Baroody 1999).

The nasal vestibule, opening to the outside environment, possesses numerous nasal hairs (vibrissae) that filter large air-borne particles. The epithelial cells in this region are stratified, squamous and keratinized with sebaceous glands. Due to its nature, the nasal vestibule is very resistant to dehydration and can withstand insults from noxious substances of the environment. On the other hand, permeation of substances through it is very limited. As a result, it is not the preferred site for drug administration and absorption. The intermediate region between the nasal vestibule and nasal conchae is the atrium. This is a transitional epithelial region with stratified, squamous cells anteriorly and pseudostratified columnar cells with microvilli posteriorly. Pseudostratified columnar epithelial cells (Figure 2) interspersed with goblet cells cover the respiratory region (the turbinates), and also present are seromucus ducts, the openings of subepithelial seromucus glands. Furthermore, many of these cells possess actively beating cilia with microvilli. Each ciliated cell contains approximately 100 cilia, and both ciliated and nonciliated cells possess approximately 300 microvilli each. Also present are nonciliated cells and basal cells. The basal cells subsequently differentiate to form other epithelial cell types and are also believed to help the columnar cells adhere to the basement membrane (Mygind & Dahl 1998). Collectively, the epithelium and lamina propria are called respiratory mucus membrane or respiratory mucosa (Burkitt et al 1993). The respiratory mucosa is the region where drug absorption is optimal. A thin sheet of mucus produced from the seromucus glands and goblet cells covers the nasal turbinates and the atrium.

Sensory innervation and nervous system control. Nasal blood supply and secretion are controlled by the autonomic nervous system. Sensory innervation of the nasal cavity is via the ophthalmic and maxillary divisions of the trigeminal nerve (Babin 1977). The resistance vessels (capillaries), located close to the surface of the nasal mucosa, are muscular vessels with narrow lumen. These

vessels are predominantly under α-adrenergic control but also receive α-adrenergic innervation, and provide the blood needed to heat and humidify inspired air. The capacitance vessels (venous sinusoids) are thin-walled and elastic, and are located deeper within the submucosa. They receive primarily α-adrenergic innervation. The capacitance vessels are responsible for most of the blood content of the nasal mucosa.

Both parasympathetic fibres and sympathetic fibres innervate nasal secretory glands. The stimulation of parasympathetic fibres causes increased secretion that is proportional to the frequency of stimulation, and is blocked by atropine. It also slowly dilates the capacitance and the resistance vessels leading to increase in total nasal blood flow, and this effect is not affected by atropine. Sympathetic stimulation causes a strong and rapid contraction of the resistance vessels, decreased capacitance blood flow, decreased nasal airway resistance, and a reduction in total nasal blood flow (Babin 1977; Baroody 1999).

Nasal secretion and mucus layer. A blanket of viscoelastic fluid, the mucus, covers the respiratory part of the nasal cavity. The greater quantity of nasal mucus is secreted from the submucosal glands. These glands are composed of both mucus cells (which secrete the mucus gels) and serous cells that produce a watery fluid (Lansley 1993). There are an estimated 100000 seromucous glands in the human nose (Tos 1983). This number is higher than in the trachea and is independent of age (Tos 1983). Mucus is also released from the goblet cells, as mucus granules. Following swelling in the nasal fluids, the mucus layer is formed. The nasal secretion is a complex mixture of several materials and consists of approximately 95% water, 2% mucin, 1% salts, 1% of other proteins such as albumin, immunoglobulins, lysozyme and lactoferrin, and < 1% lipids (Kaliner et al 1984). The production of immunoglobulin A by both the adenoid tissue and the nasal mucosa plays a very important role in immune protection against bacteria and viruses (Bernstein et al 1997). The mucus glycoproteins consist of a protein core with oligosaccharide side chains crosslinked by disulphide bridges and hydrogen bonds. Heterogeneity exists between the cytochemical characteristics of mucus secretion from seromucus glands and goblet cells (Thaete et al 1981). Approximately 1.5–2 L of mucus is produced daily (Marom et al 1984; Chien et al 1989). This mucus blanket, which is approximately 5 μm thick, is made of two layers, a lower sol layer and an upper gel layer. The lower layer, which bathes the cilia, is of low viscosity, whereas the upper gel layer that rests on the cilia is a high viscosity fluid. Consequently the viscosity of both layers would affect ciliary beating and the transport of the overlying mucus, the mucociliary clearance (MCC). The viscosity is very sensitive to even small changes in the mucin content. A small increase in mucin causes a very large increase in mucus viscosity with a resultant prolongation of the mucociliary clearance time (Rice 1978).

Mucin is a high molecular mass (2000000–4000000 Da) glycoprotein crosslinked with disulphide bridges, ionic bonds and physical entanglements. The carbohydrate side groups attached to the protein backbone include galactose, L-fucose, *N*-acetylglucoseamine, *N*-acetylgalactoseamine and *N*-acetylneuraminic acid (sialic acid). The carbohydrate side chains terminate with a sialic acid or L-fucose group, which make mucin an anionic polyelectrolyte at neutral pH. Due to the multiplicity of hydroxyl groups of the carbohydrate side chains, mucin easily forms hydrogen bonds with other suitable polymers (Kamath & Park 1994).

The nasal mucus performs a number of physiological functions (Chien 1995): it covers the mucosa, and physically and enzymatically protects it; it acts as adhesive and transports particulate matter towards the nasopharynx; the mucus has water-holding capacity; it exhibits surface electrical activity; and it permits efficient heat transfer.

Mucociliary clearance

One of the functions of the upper respiratory tract is to prevent noxious substances (allergens, bacteria, viruses, toxins etc.) from reaching the lungs. When such materials adhere to, or dissolve in, the mucus lining of the nasal cavity, they are transported towards the nasopharynx for eventual discharge into the gastrointestinal tract (GIT). Clearance of this mucus and the adsorbed/dissolved substances into the GIT is called the MCC. As the name implies, effective/efficient MCC has contributions from both the mucus and the cilia, which is the motor of the MCC. Consequently, factors that affect either the mucus or the cilia would influence the MCC. It is of utmost importance that the MCC is not impaired in order to prevent lower respiratory tract infections. The depressant effect of anaesthetics on MCC has been proposed to be the major cause of post-operative respiratory tract infections (Raphael et al 1996a, b). Even though it has been estimated that the mucus transport rate is 6 mm min^{-1} (Proctor 1977) there is a wide variation in MCC between different individuals, but within one subject it is fairly constant. The concept of "fast" movers and "slow" movers is well documented. This implies that there are individuals with a very fast

MCC rate and others whose MCC rate is slow (Baroody 1999). This is independent of age and sex. However, at the periovulatory period of the menstrual cycle, increased MCC occurs and was proposed to be due to the accompanying reduced mucus viscosity (Armengot et al 1990).

Factors that affect MCC. Both temporal environmental as well as disease conditions can influence MCC. All factors that can lead to increased mucus production, decreased mucus viscosity, increase ciliary beat frequency (CBF) without disrupting the metachronal wave, can increase the MCC rate. The opposite effects, as well as destruction of the viscoelastic properties of the mucus and disruption of the metachronal wave, reduce the MCC rate.

Environmental conditions such as temperature ($\pm$23°C) cause a moderate reduction of the MCC rate (Ridley et al 1992). However, Jorissen & Bessems (1995) reported a linear increase (0.6 Hz $°C^{-1}$) with temperature in CBF of nasal biopsy. Sulfur dioxide causes a concentration-dependent and significant reduction in the MCC rate (Ridley et al 1992). Cigarette smoking also decreases the MCC rate due to its influence on mucus rheology and/or reduction in the number of cilia, since it has no effect on CBF (Stanley et al 1986). The following pathological conditions of the upper respiratory tract influence MCC due to their effect on ciliary beating and/or mucus rheology. These include Kartagener's syndrome, Sjögren's syndrome, asthma, nasal polyposis, deviation of nasal septum, rhinitis, allergic rhinitis, common cold and chronic sinusitis.

The relevance of these disease conditions in nasal drug delivery cannot be over-emphasized. Pathological conditions with increased MCC rate reduce contact time of the drug with the absorptive nasal mucosa, whereas decreased MCC rate has the opposite effect. Nasal hypersecretion dilutes nasally-administered drug solutions leading to reduced concentration gradient, with a possible influence on absorption. A change in the pH of the mucus can affect the ionization of some drugs, and this can have a significant influence on nasal drug absorption.

Ciliary beat cycle and mechanism of ciliary beating. For the MCC to function efficiently as the first line of defence for the lungs, the cilia must beat in a well coordinated manner (both in phase and frequency), and this is called the metachronal wave. In this way a coordinated clearance towards the nasopharynx is ensured. In a small part of the anterior nares, the direction of MCC is forward, with clearance of mucus and deposited particles carried out by blowing and wiping the nose (Chien et al 1989).

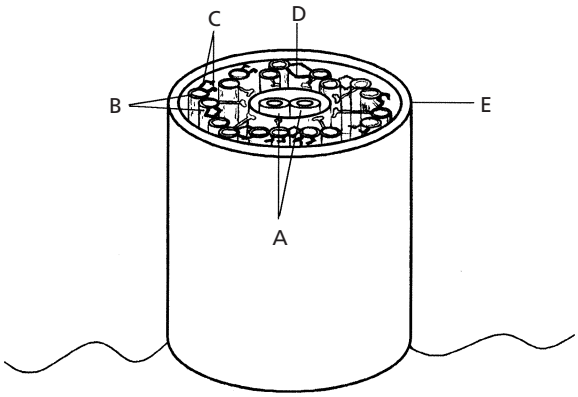

Figure 3 A cross-section of a cilium showing central doublet (A), outer doublets (B), dynein arms (C), nexin link (D) and ciliary membrane (E).

A cilium is made of an axoneme surrounded by the ciliary membrane. The axoneme itself consists of two central microtubules and nine pairs of peripheral microtubules (A and B microtubules), an arrangement termed the 9+2 formation of microtubules (Figure 3). The peripheral microtubules are connected to each other by nexin links and radial spokes connect the central microtubules to the peripheral microtubules, thereby giving the microtubule a rigid structure. Two dynein arms (inner and outer dynein) are attached to one of each pair of the peripheral microtubules. Due to their ATPase activity, the dynein arms provide the energy required for ciliary beating (Lindberg 1997).

Ciliary motility is generally accepted to result from the sliding movement of adjacent axonemal microtubules. The dynein arms of these microtubules provide the mechanochemistry for the movement, as a result of their ATPase activity. One theory of axonemal movement suggests that the dynein A microtubule transiently attaches to, and detaches from, the dynein B microtubule after ATP binding and hydrolysis, causing the doublet to move to the opposite direction. Other axonemal structures resist this movement thereby causing the bending and unidirectional movement (Lee et al 1991). The switch point theory hypothesizes that one set of the doublets is active during the effective stroke and the other set during the recovery phase. Activity therefore switches back and forth between the two sets causing the asynchronous and bending motion (Satir 1985). Another theory is that an electrochemical signal over the cell surface may be responsible for synchronizing ciliary beating in the metachronal wave, even though

this signal is not needed in initiating the ciliary beating (Guyton 1981).

Calcium ion concentration has been strongly linked with ciliary beating. Increased Ca^{2+} influx increases the beat frequency, removal of extracellular Ca^{2+} leads to a loss of ciliary beating, and addition of extracellular Ca^{2+} restores beating. Ca^{2+}-channel blockers, such as verapamil, reduce CBF (Satir & Sleigh 1990).

The cilia are also mechanosensitive appendages. In-vivo, this mechanical stimulation is provided by the overlying mucus. After Ca-ionophore A23187 treatment, mechanical stimulation did not further increase the beat frequency, even though both conditions can separately increase CBF. This suggests that both treatments increase CBF by the same mechanism, that is, increasing cytoplasmic Ca^{2+} concentration. Some sympathomimetics and parasympathomimetics (fenoterol, terbutaline, isoprenaline, methacholine) cause an increase in CBF of both human and non-human species (Wong et al 1988; Sanderson & Dirksen 1989; Agu et al 1999). The increased CBF caused by isoprenaline can be blocked by propranolol, suggesting that the effect is via α-adrenergic receptors (Verdugo et al 1980).

Ciliary beating has three identifiable phases, an active/effective phase, a rest phase and a recovery phase. During the active phase the cilium maximizes its length within the sol layer, reaching out beneath the gel mucus layer and clawing it with the tiny projections on its tip. The effective phase is followed by a rest phase when the cilium is bent and almost parallel to the cell surface. The beat cycle is completed with the recovery phase where the cilium recoils back to the initial position, ready for the next cycle. This asymmetric beating enables propulsion of the mucus in one direction. In one beat cycle each cilium makes an arc of approximately 110°. More time is spent during the rest phase than during the active or recovery phases. The frequency of ciliary beating varies a lot, with a range of 10–20 Hz.

In summary, the stimulus for CBF suggests roles for both the overlying mucus and neurotransmitters. Whereas the former increases the cytoplasmic and axonemal Ca^{2+} concentration, the latter increases intracellular cAMP. These independent mechanisms of action are supported by the finding that mechanical stimulus and application of isoprenaline have the additive effect of increasing CBF (Sanderson & Dirksen 1989).

Nasal drug delivery

Rationale for nasal drug delivery

Direct administration of drugs into the systemic circulation either by rapid intravenous bolus injection or continuous intravenous infusion is superior to other routes of drug administration with respect to the onset of therapeutic action. This stems from the lack of a lag phase in drug absorption. Due to this direct access to the general circulation, drug metabolism and degradation both in the liver and GIT (the first-pass phenomenon) is avoided. Furthermore, a constant and prolonged drug absorption period can be achieved, and the blood drug level is programmable to fall within the therapeutic range of the drug in question. The major drawbacks of intravenous administration include some potential health hazards involved during the administration, and thus it is unsuitable for outpatient use in chronic therapy, except under well-controlled conditions, such as in outpatient parenteral antibiotic therapy. It has low patient compliance. The use of both trained personnel and sophisticated equipment further drives up the cost of therapy by this method. Of course cheaper alternatives are very attractive, especially if these alternatives can duplicate the advantages of intravenous administration.

A constant rate of drug delivery can also be achieved by transdermal drug delivery. However, its major limitations include the fact that it can be used to deliver only small lipophilic and very active drugs, excluding a large number of drug candidates. There is also a long lag phase of absorption, up to several hours or more, due to the low permeability of the highly keratinized stratum corneum.

A rapid high systemic drug concentration can be achieved by intranasal administration. The last two decades have been marked by the recognition of the nasal cavity as a potential route for drug delivery. There are an ever-increasing number of research and review articles addressing various topics on nasal drug delivery. A recent review by Behl et al (1998) provided a list of 14 prescription and 33 OTC nasal products available on the market, and as many as 46 different investigational drugs that have reached the stage of human studies. This interest arises from the unique advantages presented by the nasal cavity for drug delivery purposes. These include: a relatively large surface area (epithelium covered with microvilli) available for drug absorption; a thin, porous and very vascularized epithelium with high total blood flow per cm^3, which ensures rapid absorption and onset of therapeutic action; a porous endothelial basement membrane; the direct transport of absorbed substances into the systemic circulation (or even directly into the CNS), thereby avoiding the first-pass effect attendant with peroral drug administration; lower enzymatic activity compared with the GIT and liver; amenable to self-medication, which increases patient

compliance; possibility of pulsatile delivery of some drugs such as human growth hormone; and low risk of over-dosage.

Factors that influence nasal drug absorption

A good understanding of the various factors that can influence drug absorption from the nasal cavity is very important in designing both the formulation and the device used for intranasal administration. There are several factors ranging from physiological conditions, physicochemical properties of the drug, to the administration device (Chien et al 1989; Gizurarson 1993).

Factors related to the nasal physiology include: MCC; pathological conditions such as infections, allergy, nasal obstruction, Kartagener's syndrome etc., which affect either the mucus or ciliary beating; environmental conditions (temperature, humidity); enzymatic degradation; immunology; and nasal blood flow.

Factors related to the dosage form are: physicochemical characteristics of the active ingredients; pH and mucosal irritancy; osmolarity; viscosity (solution, gels) and density (powder) of the formulation; concentration and volume administered; and type of dosage form.

Factors related to the administration device include: particle size of the droplet or powder; site and pattern of deposition; and loss from the nasal cavity after administration (drainage by gravity into the mouth or out of the nostril, and not by MCC). These factors that are related to the administration device are elaborated further.

Administration device. Drug therapy requires that administration of the dosage form be accurate and very reproducible. This therefore places stringent demands on the device for nasal drug delivery. The major mechanism of nasal deposition of particles is by inertial impaction that occurs following a change in the direction of air flow. Other contributory mechanisms are gravitational sedimentation and Brownian diffusion. Particle deposition by interception and electrostatic precipitation are of no importance in nasal deposition (Kublik & Vidgren 1998). Depending on the type of formulation, a variety of devices have been used to give drugs intranasally but mostly in experimental studies. Devices for liquid formulations include instillation catheter, droppers, unit-dose containers, squeezed bottle, pump sprays, airless and preservative-free sprays, compressed air nebulizers and metered-dose inhalers (MDIs). Devices for powder dosage forms include insufflators, mono-dose and multi-dose powder inhalers and pressurized MDIs. Delivery devices are also available for nasal gels (Kublik & Vidgren 1998). Their angle of insertion into the nostril can influence the part of the nasal cavity that the formulation comes in contact with initially and as such the overall deposition pattern.

Metered-dose nebulizers and metered-dose aerosols are superior to other devices in terms of accuracy and reproducibility (Dondeti 1996). However, the duration and condition of storage, as well as the physicochemical characteristics of the formulation such as viscosity, surface tension and homogeneity (e.g. of suspensions) affect metering accuracy. Those with manual actuation are cheaper and may be preferred due to environmental concerns. It however requires thorough and regular cleaning, is prone to bacterial contamination, and frequently require the incorporation of preservatives which could be cilio-toxic (van de Donk et al 1980; Batts et al 1989). Metered-dose nebulizers are somewhat easier to use. They rely on mechanical actuation to release the dose as a fine spray. Although the metered-dose nebulizers are known to produce less dose accuracy, it has been reported that one with metering accuracy equivalent to that of the MDIs is now available (Petri et al 1985). The particle size of aerosols is very important with regard to deposition. Particles greater than 10 μm are deposited within the upper respiratory tract, those less than 5 μm are inhaled, and those less than 0.5 μm are exhaled (Sciarra & Cutie 1990; Sanders et al 1997). Factors that influence both particle size and deposition site include: formulation factors (physicochemical characteristics of the active ingredients, particle size and shape, type and concentration of surfactant, vapour pressure and metered volume of propellant); component (device) design (actuators and adapters); and administration technique, which usually requires a very complicated series of manoeuvres (Sciarra & Cutie 1990). This problem is even made worse because very different sets of manoeuvres may be required for the devices produced by different manufacturers.

Advantages of intranasal aerosols include: delivery of a measured dose of the drug; reduced droplet or particle size; excellent deposition inside the nasal cavity with minimal inadvertent delivery into the lungs; maintenance of dose-to-dose sterility; higher patient compliance; reduced mucosal irritability; and greater flexibility in product formulation (Sciarra & Cutie 1986).

Hughes et al (1993) compared deposition and clearance of ^{99m}Tc-labelled sulfur colloids after intranasal administration to monkeys from compressed air nebulizer, nasal pump spray, drops from an instillation catheter and dry powder insufflator. In spite of the different particle sizes produced by these devices, similar

deposition patterns and clearance rates were obtained. Newman et al (1994) studied four modes of administration (80–160 μL volumes administered at gentle or vigorous inhalation air flows) and clearance in man. The administered volume influenced deposition area, but the clearance rate was not affected. A novel drug delivery device was introduced by Corbo et al (1988) for controlled nasal drug delivery. When tested on rabbits, this inflatable device could maintain high plasma progesterone level for up to 6 h, following a plateau that was achieved after 20–30 min.

The choice of a device for nasal drug delivery should take into account cost and ease of use by the patient, accuracy and reproducibility of dosing and deposition as well as clearance from the nasal cavity, since all these are critical factors that influence nasal absorption. Technical aspects of criteria for choosing a particular device should take the following factors into account: physicochemical properties of the drug in the dosage form that can be delivered from a particular device; and device performance with respect to accuracy and reproducibility of dosing as well as resistance to microbial contamination. The device should be compatible with the formulation components. Leaching of the device materials into the formulation or adsorption/absorption of formulation ingredients into the device should be avoided. Leaching of the device materials could introduce toxic substances into the formulation. Adsorption/absorption of the active ingredients leads to under-dosing, whereas adsorption/absorption of the excipients can cause formulation instability or microbial contamination. Adsorbed/absorbed materials can also alter elastomeric seal performance. Another important factor is the integrity of the device, which should be maintained throughout the shelf-life of the product. Although this particular problem can be encountered with pump delivery systems, it is more common with the pressurized aerosol systems due to the use of volatile propellants. The propellants could leak through the closure, between the valve and the container, or through the component parts of the valve. A change in propellant composition causes alteration in the performance of the device, both in terms of spray droplet characteristics and dose delivered.

Limitations of nasal drug delivery

Despite the advantages of nasal drug delivery, this route of drug administration has several limitations. The limited volume of the nasal cavity restricts the total amount of the formulation that can be administered. Consequently, only low-dose drugs or drugs with high aqueous solubility can be given intranasally so as not to adversely influence the normal physiological functions of the nasal cavity, especially with respect to olfaction and conditioning inspired air. Nasal absorption of many drugs, especially large hydrophilic molecules such as peptides and proteins, is limited. Even though the molecular mass cut-off for drug absorption from the nasal cavity (approx. 1000 Da) is higher than that of the GIT (approx. 500 Da), this threshold still excludes many potentially useful drug candidates. Since absorption is a prelude to therapeutic effect, absorption of a drug from the nasal cavity should be high enough to achieve the therapeutic objective. Furthermore, the nasal cavity is an enzymatic barrier. Nasal mucosal proteolytic enzymes have been reported to reduce nasal absorption of peptides (Hussain et al 1985, 1990b, 1992; Stratford & Lee 1986). Thus it is important to know if the drug being formulated as a nasal drug delivery system is inactivated by nasal mucosal enzymes, and possibly ways to overcome this inactivation. There should be minimal enzymatic degradation of the drug. Where this is not possible, a specific enzyme inhibitor can be incorporated in the formulation (Hussain et al 1990a). This should be achieved without undue adverse effects on the nasal mucosa.

The nasal MCC is yet another limitation of nasal drug delivery. This natural defence mechanism of the lower respiratory tract against infection and other noxious substances severely limits the time allowed for absorption to occur. Substances administered intranasally are rapidly cleared from human nose, with a clearance half-life of approximately 21 min (Soane et al 1999). The consequences are not only limited to the time allowed for absorption, it also rules out the possibility of controlled nasal drug delivery, with its well known advantages. Irregular deposition of nasally-instilled substances, which can frequently occur, is yet another limitation. Nasal deposition is influenced by a myriad of factors, such as the type of formulation, administration device, particle size of the droplets or powder (which is also influenced by the device), and health status of the nasal cavity. It is important to have a predictable deposition, preferably within the turbinate region, for optimal absorption to occur. Deposition at the vestibule may prolong the nasal residence time but absorption is low, deposition within the turbinates has the opposite effects, and deposition within the atrium is intermediate. Intranasal deposition at the olfactory epithelium may facilitate direct transport of the drug into the brain, but, for susceptible drugs, could increase enzymatic degradation.

The nasal mucosa is very sensitive to irritations. Therefore toxicological considerations are a major limi-

tation in the choice of drugs for nasal administration, formulation approaches, as well as what excipients can be used. The nasal toxicity of drugs and excipients should be well investigated, especially for drugs to be used in the management of chronic diseases.

Given the advantages and limitations of nasal drug delivery, the drug candidate for nasal administration should possess a number of attributes (Behl et al 1998): appropriate aqueous solubility and nasal absorption characteristics; minimal nasal irritation; suitable clinical rationale, such as rapid onset of action, for its formulation as a nasal drug delivery system; low dose, generally less that 25 mg; no toxic metabolites; no offensive odour or aroma; and suitable stability characteristics.

Experimental models used in nasal drug delivery studies

Many different models are used in nasal drug delivery research. These include in-vitro, in-situ and in-vivo models. Each model has its advantages and limitations that affect how the results obtained can be interpreted and/or extrapolated to the in-vivo human situation. In-vitro models are cheap and allow rapid screening of a large number of compounds, as well as avoiding the sometimes controversial use of animals in biomedical research. In-vivo models are more expensive and also labour-intensive. Variability in results can be introduced due to species differences in the animals used (in-vitro, in-situ and in-vivo). A brief description of each model would be appropriate to understand nasal drug delivery experimental methodologies.

In vitro/ex vivo models

The advantage of in-vitro approaches to experimental studies is largely due to the ability to control a lot of variables. Mechanistic aspects of nasal drug delivery are easier performed in-vitro. It is also easier to separate the process of drug permeation across the epithelium from subsequent events such as biodistribution and elimination, as well as local blood flow (Schmidt et al 1998). Other advantages of in-vitro models used in studying nasal drug delivery include: fast assessment of the potential permeability and metabolism of a drug; rapid screening of toxicity of drugs and excipients; the opportunity to elucidate the molecular mechanisms of drug transports across the epithelium and pathways of degradation and ways of preventing such degradation; the possibility of using human tissues; and a reduction in the number of animals used at later stages of the drug development process. However, the simplistic environmental conditions are frequently very different to in-vivo situations (figure 4).

A number of in-vitro models have been, and are being, developed for use in nasal drug delivery studies. These include cell cultures (both human and animal primary cell cultures and cell lines) and excised tissues (ex-vivo) from different animal species. Three types of cell lines used in nasal drug delivery studies are RPMI 2650 (derived from cancerous human septum), BT (obtained from normal bovine turbinate) and NAS 2BL (from rat nasal squamous carcinoma) (Audus et al 1990). Research with these cell lines is still at the developmental stages. BT cells do not express perijunctional complexes (tight junctions) but rather expressed stress fibres basolaterally to enhance cell attachment. RPMI 2650 expressed tight junctions but without achieving confluency (Werner & Kissel 1996). Although metabolic studies can be performed with these cell lines, their characteristics need further improvement before use in transport experiments. Human nasal epithelial cells in primary culture that attain confluency and express tight junctions, thus suitable for both transport and metabolic studies, have been reported (Kissel & Werner 1998). Ex-vivo models use materials from different animal species. The excised tissues can be mounted in Ussing chambers for performing both transport and metabolic studies.

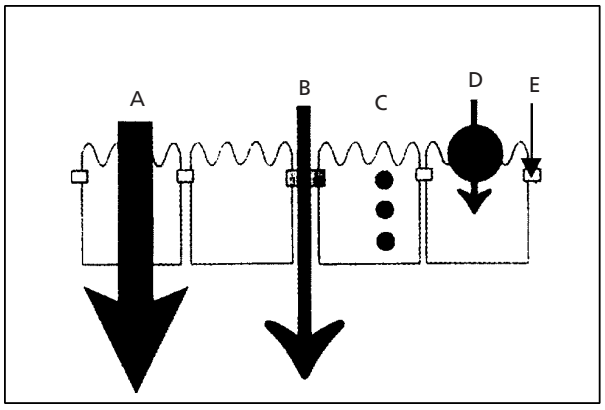

Figure 4 Drug transport pathways across the epithelium. Passive transcellular transport (A), paracellular transport (B), transcytosis (C), carrier-mediated transport (D), and intercellular tight junction (E).

In-situ models

This method involves perfusing a drug solution through the nasal cavity of an experimental animal and moni-

Table 1 Comparative anatomy of the nasal cavities of some animals and man (Chien et al 1989; Gizurason 1993).

Animal	Weight (kg)	Naris cross-section (mm^2)	Bend in naris (°)	Length (cm)	Greatest vertical diameter (mm)	Surface area (cm^2)	Volume (mL)	Bend of nasopharynx (°)	Turbinate complexity	Volume administered (μL)	Clearance half-life (min)
Rat	0.25	0.77	40	2.3	9.6	10.4–14	0.4	15	double scroll	13	5
Rabbit	3	3.1	38	4.7	25	61–90	6	45	branching	58	10
Dog (beagle)	10	16.7	30	10	23	220.7	20	30	branching	207	20
Rhesus monkey	7	22.9	30	5.3	27	61.6	8	80	single scroll	58	10
Man	70	140	–	7–8	40–45	160–181	16–19	61–90	single scroll	150	15

toring its absorption. First described by Hirai et al (1981), various modifications of this in-situ perfusion technique have been made, including a non-surgical technique by Dondeti et al (1994). Basically the in-situ technique involves anaesthetizing the animal, for example a rat, cannulating the trachea from an incision made in the neck and ligating the oesophagus. A drug solution in a reservoir is pumped through the nasal cavity, into a funnel and back to the reservoir, to be circulated again. Variations of the method exist where the reservoir is not re-circulated. Drug absorption kinetics can be followed by monitoring the changing drug concentration in the reservoir. Pharmacokinetics of the drug can also be studied by blood sampling.

This method gives detailed information about drug absorption kinetics through the nasal epithelium. However, it has several limitations. Factors such as recirculation speed, volume of circulating solution and total surface area of the nasal cavity covered by the solution all influence the results. Furthermore, the mucus covering the epithelium is gradually eroded by the circulating solution thereby exposing the epithelium and increasing the drug absorption rate. The increased drug absorption may be due to the absence of mucus barrier or the direct influence of the drug on the barrier properties of the epithelium. The nasal drug residence time is very prolonged and would give a false impression of improved absorption or increased toxicity and metabolism, unlike in-vivo situations where a short residence time is obtained. Additionally drug absorption from powders, gels and ointments cannot be studied using the in-situ perfusion model. These limitations necessitate careful evaluation of such results before extrapolation to in-vivo situations.

The usefulness of the in-situ model has been demonstrated with studies on drugs with different physicochemical properties, such as salicylic acid, aminopyrine, sulbenicillin, insulin, cefazolin, cephacetrile and phenol red (Hirai et al 1981), ergotamine tartrate (Hussain et al 1984), leucine enkephalin (Hussain et al 1985), sodium benzoate, sodium barbital, sodium phenobarbital, sodium pentobarbital, sodium secobarbital, L-tyrosine and propranolol.HCl (Huang et al 1985a, b), hydralazine (Kaneo 1983), insulin and polyethylene glycol (Kotani et al 1983), and hydromorphine (Chang et al 1988).

In-vivo models

Nasal absorption studies have been performed using different animal species. These studies allowed both pharmacokinetics and pharmacodynamics of the drug to be evaluated, which is not possible with the above models. The diversity in animal species can however complicate interpretation and comparison of results, and their extrapolation to the human situation. Permeability and metabolic capacity of the nasal mucosa of different animal species may differ. Differences in the nasal anatomy would influence drug deposition and distribution, and as such nasal absorption. However, the cost of acquiring and keeping these animals has frequently been the determining factor for the choice of which to use.

Table 1 presents an interspecies comparison of the anatomical characteristics of the nasal cavities (Chien et al 1989; Gizurason 1993). Mouse, rat, guinea-pig, hamster, rabbit, dog, sheep and monkey are all used in nasal drug delivery experiments. The limited volume that can be administered presents formulation challenges particularly for poorly-soluble drugs, especially as the volume administered can influence drug absorption.

The hamster is not the animal of choice for nasal absorption studies. At some regions of its nasal cavity, the epithelium is multilayered and this makes the drug

absorption results difficult to compare with other animal species or even to extrapolate to the human situation.

The mouse is largely used in intranasal immunization and toxicity studies. Pharmacokinetic studies are limited due to its small size, and consequently the small total blood volume. The respiratory epithelium is composed of pseudostratified columnar epithelium that is ciliated. The percentages of mouse nasal cavity covered by squamous, pseudostratified columnar and olfactory epithelium are 7, 46 and 47 %, respectively. For nasal toxicity experiments, mice cannot be used as intra-animal controls because of the existence of a septal window. This connects the two nasal cavities and allows inter-cavity migration of substances under study (Chandler et al 1991a).

The nasal cavity of rats has three regions of distinct epithelial types: squamous, pseudostratified columnar and olfactory epithelium. The relative proportions of the total surface area of the nasal cavity they cover are 3, 47, and 50 %, respectively, which is similar to that of mouse. The nasal septal window is also present, with the aforementioned disadvantage. Furthermore, the location of the nasopalatine tract, anteriorly in the nasal cavity, has a consequence that nasally-administered drug solutions can easily drain into the oral cavity. Hussain et al (1980) suggested that it is important to close the palatine during drug absorption studies. The orifice (entrance) of the rat nose is restricted (Table 1) such that drug administration could be difficult, especially for powder formulations. The advantages favouring the use of rats in nasal drug delivery studies are the ready availability and low unit cost.

The guinea-pig is also used mostly for immunization studies. The septal window is also present, and squamous epithelium covers a large part of the vestibule such that drugs have to be applied deep into the nasal cavity (Gizurason 1993).

The use of rabbits offers a number of advantages. They are suitable for both pharmacokinetic and pharmacodynamic studies. Additionally, repeated experiments can be performed on the same animal after an appropriate drug washout and recovery period. This would reduce both the total costs of experiments and variability in the data. The larger entrance into the nasal cavity (orifice) compared with rats enables easy administration of both drug solutions and powders. Pharmacokinetic experiments can be performed without the need for anaesthesia, especially since some anaesthetics can inhibit MCC (Raphael et al 1996a, b), which would affect nasal drug absorption. Other advantages include the relatively cheap price, easy availability, maintenance and handling. The large total blood volume (approx. 300 mL) can allow repeated blood sampling. The branching nasal septum of rabbits is anatomically similar to dogs. The respiratory mucosa is pseudostratified and columnar ciliated epithelium with goblet cells.

The sheep presents a nasal cavity with surface area and volume that is even larger than that of man. Its docile nature, which facilitates easy intranasal administration, is a major advantage. Pharmacokinetic and pharmacodynamic studies can be repeatedly performed on it without anaesthesia. It is however more expensive than smaller animals such as rats and rabbits. During warm weather conditions, the insulation provided by the wool prevents evaporation of sweat from the skin. The nasal cavity provides the route for sweat evaporation under such conditions. This could influence nasal drug absorption, especially of drugs transported across the epithelium via passive paracellular transport. This calls for proper housing conditions during experiments (Gizurason 1993).

Dogs can also be used easily without anaesthesia in nasal drug delivery studies. Both pharmacokinetic and pharmacodynamic studies are also possible, and can be repeated on the same animal. Some breeds of dogs are easy to handle during intranasal administration. The large nasal cavity is very interesting but cost is a major limitation. Cost considerations rule out the routine use of sheep and dogs, but not rabbits, for nasal toxicity studies.

The only advantage of using monkeys in drug research in general is that they are primates. However, they are very expensive and their use in medical research is strongly protested against by animal rights groups.

While the use of animals in medical research continues to be necessary, the choice of which animal to be used depends on the study in question. For nasal drug delivery purposes, small laboratory animals (mice, rats, and guinea-pigs) are very useful for toxicity studies due to their cheap price. Larger animals (dogs, sheep) can be used repeatedly for pharmacokinetic and pharmacodynamic experiments. Rabbits can be used conveniently for both types of experiments.

Enhancement of nasal drug absorption

Mechanism of absorption enhancement

There are many hypothetical mechanisms proposed for absorption enhancement. This is understandable due to the large number of chemically-unrelated classes of compounds used in absorption enhancement. The major mechanisms proposed include alteration of the properties of the mucus (O'Hagan et al 1990), inhibition of ciliary beat frequency, (Morimoto et al 1991a, b), en-

hancement of both transcellular and paracellular transport (O'Hagan et al 1990; Uchida et al 1991) with the latter as a result of the influence on tight junction regulation (Leußen et al 1994; Ganem-Quintanar et al 1997), enzyme inhibition (Morimoto et al 1991b; Leußen et al 1994), influence on the thermodynamic activity of the drug (Uchida et al 1991), and increasing fluidity of the membrane lipid bilayer (Ganem-Quintanar et al 1997). Some of the proposed mechanisms of many absorption enhancers are reviewed by Arnaud (1993).

Mucolytic agents alter mucus rheology, making it more permeable to drug molecules. The nasal mucus is negatively charged. Alteration of its charge characteristics could potentially increase or decrease absorption of a charged molecule. Mutual attraction could increase local residence time, whereas repulsion has the opposite effect. Absorption enhancement due to an effect on CBF (i.e. cilio-inhibition) results in increased local drug residence time. Substances that reversibly alter membrane bilayer fluidity increase transcellular transport, whereas enhancers that disrupt the mechanism of tight junction regulation increase paracellular transport. It is very important that the effects of substances that alter tight junction and membrane lipid bilayer fluidity, or extract membrane proteins, be transient and completely reversible to allow proper cell/epithelial barrier functioning, more so when the enhancers are used chronically.

As mentioned above, the nasal mucosa is also an active enzymatic barrier. Protease inhibitors have been used to increase the nasal bioavailability of many drugs. Both exopeptidases and endopeptidases, which cleave peptides and proteins at the N- and C-terminal peptide bonds and at internal peptide bonds respectively, are present in the nasal mucosa.

The thermodynamic activity of drugs is influenced by components of the vehicle used, since this influences its solubility as well as ionization, both of which have influence on absorption. Enhanced thermodynamic activity of human growth hormone was proposed to be one of the mechanisms of its enhanced absorption by sodium taurodihydrofusidate (STDHF) after intranasal absorption in rats. At the critical micelle concentration (0.16%) there was a marked increase in absorption; above 0.5% concentration, nasal absorption started decreasing even though these concentrations were associated with more mucosal damage (Baldwin et al 1990).

A number of approaches are used to counter the various limitations of nasal drug administration. The three major approaches that have been attempted are: the use of chemical enhancers to improve absorption; incorporation of enzyme inhibitors; and increasing drug local residence time using mucoadhesive polymers (Ugwoke 1999).

Mucoadhesive drug delivery

An alternative approach to the use of chemical enhancers to improve nasal drug absorption is to increase the duration of formulation residence within the nasal cavity. This is achieved by the use of bioadhesive polymers. Bioadhesion is the ability of a synthetic or natural material to adhere to a biological tissue for a prolonged period of time (Longer & Robinson 1986). This encompasses a wide variety of adhesion possibilities obtainable in nature. These include: adhesion between two normal cells; adhesion of cells to foreign materials; adhesion of normal cells to pathological cells; and adhesion of an adhesive (polymer) to a biological tissue (Baier 1982; Gayot 1985; Longer & Robinson 1986). Bioadhesion in drug delivery implies the attachment of a drug delivery system to a specific biological tissue, which increases the local residence time of the delivery system. Mucoadhesion means specifically that the adhesive (mucoadhesive) interacts with the mucus covering of a biological tissue in such a way that the local residence time is prolonged. It involves interaction between mucin and a synthetic or natural polymer leading to a net attraction (Leung & Robinson 1990).

Apart from these synthetic and natural polymers, there is now a new class of promising compounds, the lectins, often referred to as second generation mucoadhesive materials. These are non-immunogenic proteins or glycoproteins capable of specific recognition and reversible binding to carbohydrate moieties of complex glycoconjugates without altering the covalent nature of any of the recognized glycosyl ligands (Sharon 1993). Although these lectins have yet to be investigated for use in nasal mucoadhesive drug delivery, they have proved very useful as drug targeting agents, enhancing drug delivery to the GIT (Irache 1994; Naisbett & Woodley 1995a, b; Ezpeleta et al 1999), and ocular cavity (Nicholls 1996). Interestingly, some lectins have also been reported to show very low toxicity after intradermal injection (Smart et al 1999). Given the specificity of binding of lectins to sugar residues and the recent finding that uptake of lectin-coated nanoparticles is dependent on the density of the coating but not on the type and size of lectin (Russell-Jones et al 1999), it could be predicted that lectin-mediated mucoadhesion would be very useful for nasal drug delivery. This is especially true because excessive formulation dilution obtained after oral administration is lower with nasal drug delivery systems.

The use of mucoadhesives can solve a number of problems encountered in controlled drug delivery. It localizes the formulation at a particular region in the body, thereby improving the bioavailability of drugs with low bioavailability. The increased contact time and localization of the drug due to the strong interaction between the polymer and mucus is essential for the modification of tissue permeability. Furthermore, enzymatic activity can be locally inhibited to improve the bioavailability of drugs that are subject to enzymatic degradation. This has been demonstrated for some mucoadhesive polymers such as Carbopol 934P and polycarbophil that inhibit the proteolytic enzyme trypsin, which can thus increase the stability of co-administered peptides (Leußen et al 1994). Some studies have also demonstrated that mucoadhesive polymers can also directly interact with the epithelial tight junctions by increasing their permeability to administered drug molecules (Leußen et al 1994), an effect analogous to those of chemical absorption enhancers. Agents can be freed locally to modulate antigenicity (Jiménez-Castellanos et al 1993). The mucus coat covering a number of anatomical regions in the body provides opportunities for the mucoadhesion approach to drug delivery via buccal, sublingual, vaginal, rectal, ocular, gastrointestinal and nasal routes.

To improve bioavailability, buccal and sublingual drug administrations have the advantage of avoiding GIT and hepatic degradation and metabolism. Unfortunately, it is usually difficult to retain a tablet at a particular location in the mouth, or even not to swallow it, a problem that can be solved with mucoadhesive buccal or sublingual tablets. The GIT transit time limits the time allowed for oral absorption of all drugs. Therefore oral controlled release drug delivery devices can be improved if they can be localized within the GIT for periods longer than the GIT transit time. Drugs with an absorption window, if formulated as a mucoadhesive delivery system capable of adhering to the absorbing site, would be a big advantage. Retention of drugs in the lower rectum is one approach to avoid the hepatic first-pass effect. This can also be achieved by mucoadhesive drug delivery. Vaginal drug administration, both for local and systemic effects, would also profit by retention of the drug within the vaginal cavity with subsequent prolonged drug release without the messiness usually associated with many vaginal formulations. In ocular drug delivery, less than 2 min is required for the clearance of instilled solutions, and so mucoadhesive drug delivery is particularly appealing. Similarly nasally-administered substances are rapidly cleared by MCC. Nasal mucoadhesive drug delivery has been under active investigation due to the peculiar advantages of the nasal cavity, in particular the opportunity to formulate controlled-release dosage forms (Ugwoke 1999; Ugwoke et al 1999a, b). The types of polymers used to improve the nasal bioavailability of several drugs and the levels of improved bioavailability obtained have been comprehensively reviewed elsewhere (Kamath & Park 1994; Dondeti et al 1996).

Several theories have been put forward to explain the mechanism of polymer–mucus interactions that lead to mucoadhesion. The sequential events that occur during bioadhesion include an intimate contact between the bioadhesive polymer and the biological tissue due to proper wetting of the bioadhesive surface and swelling of the bioadhesive. Following this is the penetration of the bioadhesive into the tissue crevices, interpenetration between the mucoadhesive polymer chains and those of the mucus. Subsequently low chemical bonds can become operative (Duchêne et al 1988; Jiménez-Castellanos et al 1993).

Factors that influence mucoadhesion

Factors that influence the mucoadhesiveness of a polymer include the type of functional groups, polymer molecular mass, molecular mass between crosslinks (crosslinking density), spatial orientation, contact time with mucus, polymer concentration, environmental pH and physiological variables such as mucin turnover and disease conditions. These will be further explained under the subheadings, polymer-related, environment-related and physiological-related factors.

Polymer-related factors

Polymer type, molecular mass, crosslinking density and spatial orientation. The chemical class of a polymer has a strong influence on its mucoadhesive properties. Using rheological synergism as an index of mucoadhesion, Madsen et al (1998a, b) reported that the polyacrylic acids (Noveon and Carbopol) and carageenan, showed much higher rheological synergism compared with alginate, xanthan gum, CMC, HPMC and polyethylene oxide. The observation was attributed to the polymer types. Polymer molecular mass influences its bioadhesion characteristics. There is a critical polymer molecular mass below or above which there is reduced mucoadhesive power, and this is dependent on the type of polymer. Tobyn et al (1996) reported that the mucoadhesive power of polyacrylic acid increased with increasing polymer molecular mass up to 750000 Da, beyond which a decrease was seen. Gurny et al (1984) also reported increased bioadhesive power with increas-

ing molecular mass up to 1 000 000 Da, beyond which no significant increase in mucoadhesive strength resulted. On the other hand, the bioadhesiveness of polyethyleneoxide increased with an increase in molecular mass up to 4 000 000 Da. The optimum molecular mass for bioadhesion of sodium carboxymethylcellulose was $\geqslant$ 78 600 even though mucoadhesion increased with increasing molecular mass (Smart et al 1984). Increasing both the molecular mass and crosslinking density reduced the bioadhesive power of carbomer (Blanco-Fuente et al 1996). Mucoadhesion requires an adequate free chain length for interpenetration, which is provided by the linear structure of the aforementioned polyethyleneoxide. Reducing the free chain length by extensive crosslinking reduces mucoadhesion. This was observed with polyacrylic acids (Park & Robinson 1987; Tobyn et al 1996).

Another important factor is spatial orientation of the polymer chains. This has been used to explain the poor bioadhesion characteristics of dextran of molecular mass up to 19 500 000 Da, which has similar adhesive strength as PEG of molecular mass 200 000 Da (Jiménez-Castellanos et al 1993). The helical structure of dextran is thought to shield most of the active sites used for adhesion inside the coils thereby reducing its bioadhesive power.

We recently reported the in-vivo nasal mucoadhesive capability of two polymers, CMC and Carbopol, and found that CMC was cleared faster than Carbopol even up to 6 h after administration in rabbits (Ugwoke et al 2000a).

Polymer concentration. There is an optimum polymer concentration required at the polymer–mucus interface for bioadhesion, beyond which few polymer chains are available for polymer–mucus interpenetration. This requirement may be relevant only for bioadhesion in the gel form (Gurny et al 1984). A higher polymer concentration of bioadhesive tablets resulted in greater bioadhesive power (Ponchel et al 1987a, b; Duchéne et al 1988). Hagerstrom et al (2000) also reported a concentration-dependent rheological interaction between mucin and the polymers, Carbopol and Gelrite.

Environment-related factors

Hydration condition and swelling. These two factors are related both to the polymer and the environment. As mentioned previously, an excessive amount of hydration fluid leads to inordinate swelling of the polymer, which reduces its adhesive strength. A compromise should be reached between the swelling, particularly the rate of swelling, and hydration. It is important that the swelling–hydration rate should not be too rapid in order to prolong the adhesion time. However, inordinate swelling is eventually required to reduce polymer adhesiveness and to allow it to detach from the biological tissue.

Environmental pH. The environmental pH affects bioadhesion due to its influence on the charge characteristics of the mucus and polymer depending on its type. Due to dissociation of functional groups of the mucus glycoprotein, different charge distributions exist depending on the pH. The maximum adhesion of ionizable polymers such as polycarbophil occurred at pH 3 and below (Park & Robinson 1985) when the polymer is unionized, favouring hydrogen bonding between the protonated carboxyl group and the mucin molecules. Increasing the pH sharply decreased the bioadhesive strength with practically no adhesion at pH 6 and 7. Increasing the pH neutralizes the polycarbophil. The charged carboxyl groups repel each other leading to increased polymer swelling. Additionally, there is repulsion of the negatively-charged groups of mucin with a resultant reduction in mucoadhesion. This hypothesis was supported by the work of Tur & Ch'ng (1998) who reported that both polyacrylic acid and polymethacrylic acid showed the strongest mucoadhesion using a texture analyser in saline, the least in simulated intestinal fluid, with simulated gastric fluid being intermediate. Madsen et al (1998a) also reported the effect of pH on the rheological synergism for polyacrylic acids (Noveon and Pemulen), carageenan and CMC. Whereas CMC was not affected by pH, the polyacrylic acids and carageenan showed maximum rheological synergism at weakly acidic pH values (pH 5.2 for polyacrylic acids; pH 6.2 for carageenan). Satoh et al (1989) reported an inter-polymer complex between Carbopol 934 and hydroxypropyl cellulose below pH 4.5 due to hydrogen bonding. For effective hydrogen bonding to occur, hydrophilic functional groups such as carboxyl, hydroxyl, amide and sulfate are required. Polyanionic polymers are preferred over polycationic polymers with regard to bioadhesiveness (Park et al 1984; Peppas 1985; Leung & Robinson 1988; Duchêne & Ponchel 1993).

Contact time and applied pressure. It has been reported that increased contact time and applied pressure increase the adhesiveness of a polymer (Smart et al 1984; Park & Robinson 1985; Leung & Robinson 1990). The polymer type also plays a part. Whereas polyhydroxyethyl-

methacrylic acid required a critical applied pressure for adhesion to occur, polyacrylic acid and polymethacrylic acid requires no applied pressure for interaction with mucin (Park & Robinson 1985). However, increasing the force applied from 2 to 10 g progressively led to increased detachment force required (Tur & Ch'ng 1998).

Physiological-related factors

MCC, mucus turnover and disease states are physiological factors which influence nasal mucoadhesion. Mucoadhesion can slow down MCC, but with time, mucus production reduces the mucoadhesion bond strength, allowing a recovery of MCC to normal clearance rates, which removes the mucoadhesive from its adhesion site. Disease conditions mentioned above can affect mucoadhesion due to their influence on either mucus production or ciliary beating. It is recommended that if a mucoadhesive is to be used in a certain disease state, its mucoadhesive capabilities should be studied under the same conditions (Kamath & Park 1994).

Safety considerations

The successful use of absorption enhancers is not only limited to their absorption enhancement efficacy. Equally important, and even more so in chronic disease states, is the safety of the absorption enhancer. The major areas of concern are local irritation of the mucosa, the effect on MCC, epithelial damage, and the rate of recovery of the damaged mucosa.

Local irritation is the mildest form of toxicity of an absorption enhancer, causing only mild discomfort that frequently elicits the sneeze reflex to quickly remove the noxious agent. Nasal irritants have been implicated in causing the release of neuropeptides such as substance P, somatostatin and calcitonin gene-related peptide from the nasal mucosal neurons, epithelial cells, mast cells and leucocytes, which may in turn induce the sneeze reflex and nasal secretion (Geppetti et al 1988; Walker et al 1988). These are similar to the changes that occur in the common cold and hay fever, the relationship of which to nasal absorption is uncertain. Larsen et al (1987) reported that histamine-induced rhinitis did not influence nasal absorption of buserelin, whereas Olanoff et al (1987) reported that the nasal absorption of desmopressin was improved after a similar treatment.

Increased nasal absorption-enhancement efficacy can be associated with epithelial damage, increased mucus discharge, squamous metaplasia, cilia erosion and epithelial necrosis. A low degree of epithelial damage can elicit an inflammatory response causing exudation of plasma and white blood cells from the blood into the inflamed tissue. Tissue injuries in general cause the release of increased quantities of histamine, bradykinin and serotonin into the inflamed tissue. These in turn cause increased local blood flow and increased capillary permeability (Guyton 1981). Soon after the onset of inflammation, macrophages, lymphocytes and neutrophils invade the tissue to destroy the noxious agent. Examining tissues by light microscopy for these agents of inflammation, particularly neutrophils, is a very good indicator of the level of mucosal tissue inflammation. Since absorption enhancers increase absorption due to their effects on epithelial membrane, some studies have tried to correlate increased absorption with tissue damage. Chandler et al (1991b) found that a ranked order for increased absorption was laureth-9 > lysophosphatidylcholine = STDHF > diethylaminoethyl-dextran $\gg$ no enhancer. This did not correlate very well with the histological damages they elicited, the ranked order of which was laureth-9 $\gg$ lysophosphatidylcholine $\geqslant$ STDHF > diethylaminoethyl-dextran > no enhancer. However it does point to a close relationship between absorption enhancement and tissue damage. Similarly, absorption enhancement efficacy for insulin by lysophospholipids in rats was lysophosphatidylcholine-decanoyl $\geqslant$ lysophosphatidylcholine-caproyl = no enhancer, whereas the degree of histological damage was lysophosphatidylcholine-decanoyl $\gg$ lysophosphatidylcholine-caproyl = no enhancer (Chandler et al 1994). Laureth-9, sodiumdodecyl sulfate, sodium deoxycholate, sodium taurodeoxycholate and STDHF all caused epithelial disruption (Daugherty et al 1988; Donovan et al 1990; Ennis et al 1990; Chandler et al 1991b).

Recently we reported severe nasal mucosal inflammation caused by Carbopol 971P after 1 week, and this increased with duration of treatment for 4 weeks when the study was terminated (Ugwoke et al 2000b). Even though no epithelial necrosis was observed, the degree of neutrophil infiltration of the lamina propria, epithelium and the lumen were too severe for this carrier to be used in man. Sodium glycholate and didecanoyl-L-α-phosphatidylcholine have no effect on MCC, whereas lysophosphatidylcholine, laureth-9, sodium dihydrofusidate, sodium desoxycholate and dimethyl-α-cyclodextrin have ciliostatic effects (Gizurarson et al 1990; Merkus et al 1991). The use of the suspension cell culture of human nasal epithelium, the only reported cell culture system of human nasal epithelium that expresses in-vitro ciliogenesis with actively beating cilia for several months (Jorissen et al 1989), has recently been validated (Agu et al 1999) and applied for the assessment of cilio-toxicity of nasal formulations and

Table 2 Classification of absorption enhancers based on their tissue reactivity (Muranishi 1990).

Class	Enhancing efficiency	Safety	Examples of enhancers[a]
I	Strong and fast reaction with tissue, with a fast recovery of functional properties	Comparatively safe	Fatty acids (capric, oleic, linoleic and arachidonic acids and their monoglycerides), acylcarnitines, alkylsaccharides (LM and OG), Azone
II	Moderate and fast reaction with tissue, with a fast recovery of functional properties	Comparatively safe	Bile salts (cholate, STDHF), salicylates (3-methoxysalicylate, 5-methoxysalicylate), homovanilate
III	Strong or moderate reaction with tissue, with a slow recovery of functional properties	Tissue disturbance remains, thus not very safe	Strong surfactants (SLS, laureth-9, Brij 35), chelating agents (EDTA, EGTA), citric acid, phytic acid, DEEMM
IV	Moderate reaction with tissue	Comparatively safe, but with possibility of systemic side effects	DMSO, DMAC, DMF, ethanol

[a]Abbreviations used in the table: Azone, 1-dodecyl azacycloheptan-2-one; Brij 35, dodecanol polyoxyethylene ether; DEEMM, diethyloxymethylene maleate; DMAC, *N*,*N*-dimethyl acetamide; DMF, *N*,*N*-dimethylformamide, DMSO: dimethylsulfoxide; EDTA, ethylene diaminetetraacetic acid; EGTA, ethylene glycol-bis(α-aminoethyl ether) *N*,*N*,*N*′,*N*′,-tetraacetic acid; LM, *n*-lauryl-α-D-glucopyranoside; OG, octyl-α-D-glucopyranoside; SLS, sodium lauryl sulfate; STDHF, sodium tauro-24,25 dihydrofusidate.

excipients (Agu et al 2000; Ugwoke et al 2000b, c). Based on the results obtained using this cell culture model, together with anatomopathological investigations in rabbits, we found carboxymethylcellulose to be well tolerated with only mild mucosal inflammation observed after a 1-month twice-daily treatment (Ugwoke et al 2000c).

Sodium deoxycholate and sodium glycocholate caused release of the membrane and cytoplasmic proteins, 5′nucleotidase and lactate dehydrogenase, respectively (Shao & Mitra 1992).

Recovery rate of the mucosa after damage is also essential in assessing the toxicity of an absorption enhancer (Table 2). Slow mucosal recovery rates were observed with laureth-9 (Richardson et al 1991; Zhou and Donovan 1996). The type and severity of nasal mucosal damage caused by many absorption enhancers are detailed in a review by Lee et al (1991).

Conclusions

Literature evidence suggests that the active interest in nasal drug delivery will continue to grow. Novel approaches to nasal drug delivery will also continue to be explored. However, the long-term use of a nasal drug delivery system will mainly depend on its local toxicity. It is very important that nasal toxicity research is increased and the results reported. In this way, toxic excipients will become known, and avoided. Appropriate design of these studies is very essential so as not to draw the wrong conclusion about the toxicity of a given formulation.

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