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Mini-review

Intranasal delivery: Physicochemical and therapeutic aspects

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Abstract

Interest in intranasal (IN) administration as a non-invasive route for drug delivery continues to grow rapidly. The nasal mucosa offers numerous benefits as a target issue for drug delivery, such as a large surface area for delivery, rapid drug onset, potential for central nervous system delivery, and no first-pass metabolism. A wide variety of therapeutic compounds can be delivered IN, including relatively large molecules such as peptides and proteins, particularly in the presence of permeation enhancers. The current review provides an in-depth discussion of therapeutic aspects of IN delivery including consideration of the intended indication, regimen, and patient population, as well as physicochemical properties of the drug itself. Case examples are provided to illustrate the utility of IN dosing. It is anticipated that the present review will prove useful for formulation scientists considering IN delivery as a delivery route.

Keywords: Intranasal drug delivery; Nasal mucosa; Pharmacokinetics; Physicochemical properties

Contents

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1. Introduction

Intranasal (IN) administration represents a viable option for local and systemic delivery of diverse therapeutic compounds (Behl et al., 1998a,b; Costantino et al., 2005; Hussain, 1998; Illum, 2000, 2003, 2004; Pontiroli, 1998; Sayani and Chien, 1996; Song et al., 2004; Wearley, 1991). The large surface area of the nasal mucosa affords a rapid onset of therapeutic effect, potential for direct-to-central nervous system delivery, no first-pass metabolism, and non-invasiveness; all of which may maximize patient convenience, comfort, and compliance. Although the nasal mucosa poses a permeation barrier to high-molecular-weight therapeutics such as peptides and proteins, the tight junctions that form this barrier to paracellular drug delivery can be reversibly and safely opened (Johnson and Quay, 2005). IN delivery is non-invasive, essentially painless, does not require sterile preparation, and is easily and readily administered by the patient or a physician, e.g., in an emergency setting. Furthermore, the nasal route may offer improved delivery for "non-Lipinski" drugs (Johnson and Quay, 2005). Due to such factors, marketed IN formulations exist for a variety of low- and high-molecular-weight drugs (e.g., peptides and proteins), and there are other products under development.

Given these positive attributes, it is logical to consider IN administration when developing new therapeutics, or when extending the life or improving the profile of an existing drug. In order to assess the desirability and viability of such an approach, a series of questions regarding the drug and its use should be addressed. Is the drug intended for local or systemic delivery? Will the drug be delivered chronically or acutely? Is the patient population needle-naïve? Are the physicochemical properties of the drug suitable for intranasal delivery and can clinically relevant bioavailability be achieved (an important aspect for peptides and proteins)? These questions are considered below in light of their impact on a drug's suitability for IN development.

2. Therapeutic considerations

Therapeutic considerations are paramount when selecting the dosing route. Such considerations include the pharmaceutical target (e.g., local versus systemic), the dosing frequency, and the patient population. In some cases, IN delivery may not only be possible, but may also be the preferred mode of administration.

2.1. Local delivery

IN is a logical delivery choice for local (or topical) treatment. Prominent examples are decongestants for nasal cold symptoms, and antihistamines and corticosteroids for allergic rhinitis (Bloebaum, 2002). Examples of nasal products with widespread use in this area include the histamine H_1-antagonist levocabastine (e.g., Janssens and Vanden-Bussche, 1991), the anti-cholinergic agent ipratropium bromide (e.g., Milford et al., 1990), and steroidal anti-inflammatory agents such as budesonide (e.g., Stanaland, 2004), mometasone furoate (e.g., van Drunen et al., 2005), triamcinolone (Lumry et al., 2003), and beclomethasone (Lumry et al., 2003).

As reviewed by Salib and Howarth (2003), IN corticosteroids and antihistamines have minimal potential for systemic adverse effects (as opposed to oral therapy), primarily due to the fact that relatively low doses are effective when administered topically. For instance, the recommended therapeutic dosage of IN antihistamines does not cause significant sedation or impairment of psychomotor function, whereas these effects may be seen upon oral dosing (for which a much larger dose is required). Such factors make IN delivery of antihistamines and corticosteroids an attractive and typically preferred route of administration, particularly if rapid symptom relief is required.

2.2. Vaccine delivery

The nasal mucosa has received some attention as a vaccination route. Presentation of a suitable antigen with an appropriate adjuvant to the nasal-associated lymphoid tissue (NALT) has the potential to induce humoral and cellular immune responses (Zuercher et al., 2002). This approach may be a particularly effective approach to achieving rapid mass immunization, for instance in children and/or in developing countries and disaster areas (Roth et al., 2003). IN immunization may lead to development of local, as well as systemic, immunity. Furthermore, vaccination via the IN route does not require a sterile product or a sterile dosing technique (a distinct advantage in developing areas of the world).

An example of an IN vaccine is FluMist®, a cold-adapted live influenza virus (e.g., Kemble and Greenberg, 2003). This product is given as one or two doses over the influenza season via a syringe sprayer. Additional examples of human efficacy testing of IN vaccines includes those tar-

geted against adenovirus-vectored influenza (Van Kampen et al., 2005), proteosome-influenza (Treanor et al., 2006), influenza A (Treanor et al., 1992), influenza B (Obrosova-Serova et al., 1990), meningococcal outer membrane vesicle (Oftung et al., 1999), and a combination respiratory syncytial virus (RSV) and parainfluenza 3 virus (PIV3) live, attenuated intranasal vaccine (Belshe et al., 2004).

Effective nasal immunization requires an effective antigen and/or a potent mucosal adjuvant or carrier. Research in this area includes exploring various IN excipients such as chitosan (Read et al., 2005), chitin (Hasegawa et al., 2005), galactoseramide (Ko et al., 2005), and biodegradable polymers (Koping-Hoggard et al., 2005). It is important to note that even for active antigens, IN delivery may not elicit an immune response in the absence of an effective adjuvant (McCluskie and Davis, 1998). In fact, it has been suggested that IN dosing can be effective for inducing nasal mucosal (Harrison et al., 2004; Mestecky et al., 2005) and oromucosal (e.g., Meritet et al., 2001) tolerance for a variety of molecules, including therapeutic peptides and proteins.

2.3. Systemic delivery

Positive attributes of IN systemic delivery include a relatively large surface area for drug absorption, rapid drug onset, no first-pass metabolism, and non-invasiveness to maximize patient comfort and compliance. Specific pharmacokinetic attributes of IN delivery are reviewed elsewhere (Costantino et al., 2005). As discussed in the various case studies below, IN administration provides an alternative route for systemic delivery of drugs more conventionally delivered by oral or (for poorly orally absorbed compounds such as peptides and proteins) injection routes.

2.4. Chronic versus acute therapeutic use

When deciding on a delivery route, it is important to consider the dosing regimen for the drug. Is the intended use acute or chronic? For an acute indication, the advantage of patient comfort and compliance afforded by IN dosing (as compared with injections) may not be a major factor. Even so, there are advantages to IN dosing in certain acute situations. One example is the case of an emergency room setting, where the avoidance of accidental needle stick potential is desired (Wolfe and Barton, 2003).

Other examples of acutely dosed therapeutics that have been explored for IN administration include epinephrine (Bleske et al., 1996) and cardiovascular agents such as nitroglycerin (Landau et al., 1994). In principal, IN administration is suitable for either acute or chronic use over a wide range of lengths of course and frequency of therapy. Dosing frequencies of current marketed IN products range from those dosed relatively infrequently, e.g., weekly dosing for Nascobal® Spray (for the treatment of vitamin B12 deficiencies), to multiple times daily, e.g., two sprays per nostril two to three times daily for ATROVENT® Nasal Spray (indicated for symptomatic relief of rhinorrhea associated with allergic and nonallergic perennial rhinitis). IN dosing may be particularly suited for the circumstance of a chronic application for a non-orally bioavailable drug to be given to a needle-naïve patient population.

2.5. CNS delivery

IN delivery of drugs targeting the central nervous system (CNS) is currently an area of great interest, as reviewed elsewhere (Illum, 2004; Vyas et al., 2005). Improved delivery to the brain via the IN route has been reported for some low-molecular-weight drugs (Sakane et al., 1991, 1994, 1995; Kao et al., 2000; Chow et al., 2001; Al-Ghananeem et al., 2002; Costantino et al., 2005; Barakat et al., 2006), as well as therapeutic peptides and proteins (Frey et al., 1997; Dufes et al., 2003; Banks et al., 2004; Thorne et al., 2004; Ross et al., 2004; Lerner et al., 2004).

However, it should be noted that there are also cases for which there was no evidence found for preferential delivery to the brain via IN dosing (van den Berg, 2005; van den Berg et al., 2004a,b; Yang et al., 2005). Therefore, the potential for preferential brain delivery for IN dosing may be drug-specific, or may depend on the study methods employed (van den Berg, 2005). In addition to the potential for "nose to brain" delivery, IN drugs can enter via a "nose to systemic circulation to brain" pathway (see Fig. 1). In this case, it is necessary for the drug to readily permeate the blood–brain barrier (BBB) from the circulation. In order for this to be achieved, the drug (or prodrug) must exhibit satisfactory passive or active transport across the tight junction barriers of the BBB. For example, an insulin transporter across the BBB has been described (Banks, 2004).

2.6. Factors related to patient population

Yet another factor in considering IN delivery for a therapeutic indication is the patient population. For example, if IN delivery is being considered as an alternative to injections, what is the patient population's experience with injections, and what is their

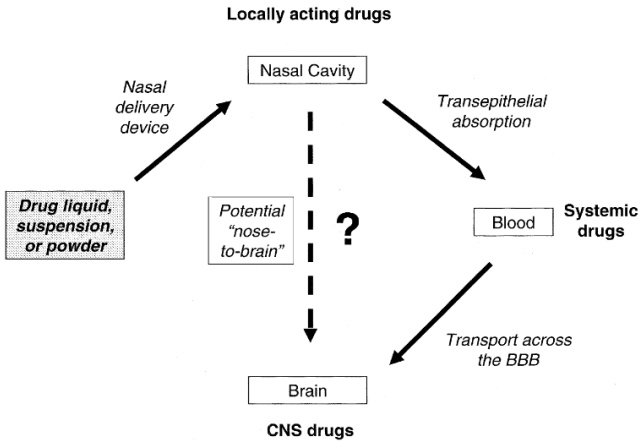

Fig. 1. Schematic of nasal drug delivery. IN drugs formulated as solutions, suspensions, or powders can be administered to the nasal cavity (local action), can transport across the epithelial tissue to enter the blood (systemic drugs), and for drugs that cross the blood–brain barrier (BBB), can subsequently enter the brain (CNS applications). Direct delivery of IN drugs to the brain has been proposed, but is not universally established in the literature.

preferred route of administration? It is believed that IN delivery is favored over injections, e.g., for insulin where a 67% patient preference was reported compared to injections (Frauman et al., 1987), although this may not always be the case, e.g., for a new intranasal fentanyl formulation where a 29% patient preference was reported (Paech et al., 2003). It is interesting to note in this context that calcitonin was first introduced as a subcutaneously delivered product, but intranasal formulations are now more widely used because of improved tolerability compared to injections (Munoz-Torres et al., 2004). As noted above, IN dosing may be particularly suited for chronic dosing to a needle-naïve patient population, as well as when oral dosing is problematic.

2.6.1. *Effect of nasal inflammation*

A common question regarding IN dosing and the intended patient population is whether inflammation of the nasal mucosa (e.g., patients with rhinitis) affects drug bioavailability. Various studies suggest that intranasal drug pharmacokinetics and/or pharmacodynamics are not affected by the presence of rhinitis. These studies include the examination of intranasal formulations of low-molecular-weight compunds (e.g., dihydroergotamine (Humbert et al., 1996), zolmitriptan (Dowson et al., 2005), and butorphanol (Shyu et al., 1993)), as well as peptide drugs (e.g., buserelin (Larsen et al., 1987) and desmopressin (Greiff et al., 2002)).

2.6.2. *Nasal physiology*

Various aspects of nasal physiology and their workings, such as nasal anatomy, airflow, resistance, and the nasal cycle (wherein the turbinates (see below) alternatively swell and congest from side to side) may have a potential impact on IN delivery. Reviews of this subject can be found elsewhere (e.g., Mygind and Dahl, 1998; Jones, 2001). Briefly, the nasal cavity is divided by the nasal septum (comprised of bone and cartilage), with each half opening at the face (via the nostrils). There is also a connection to the oral cavity provided by the nasopharynx. The anterior and posterior vestibules, the respiratory region, and the olfactory region are the three main areas of the nasal cavity. The lateral walls comprise a folded structure (refered to as the nasal labial folds or conchae). This folded structure further comprises the superior, median, and inferior turbinates, providing a total surface area of about 150 cm^2 in humans.

The epithelial tissue within the nasal cavity is relatively highly vascularized, and thus provides a potential conduit for drug delivery. The cellular makeup of the nasal epithelial tissue consists mainly of ciliated columnar cells, non-ciliated columnar cells, goblet cells and basal cells, with the proportions varying in different regions of the nasal cavity. Ciliated cells facilitate the transport of mucus towards the nasopharynx. Basal cells, which are poorly differentiated, act as stem cells to replace other epithelial cells. Goblet cells, which contain numerous secretory granules filled with mucin, produce the secretions that form the mucus layer.

2.6.3. *Variability of IN dosing*

Inter- and intra-subject variability in pharmacokinetics and/or pharmacodynamics is an important consideration when choosing the delivery route. Different administration routes should be compared (e.g., IN, oral, injection), and viable options are those with variability commensurate with the expected therapeutic window. Variability can be affected by numerous factors, including those arising from the patient, delivery device, formulation, and the drug itself. For low-molecular-weight drugs, IN dosing can provide pharmacokinetics with relatively high bioavailability and relatively low variability, which in many cases is similar to or lower than oral or even injection administration (e.g., Coda et al., 2003). However, for high-molecular-weight drugs such as peptides and proteins, IN pharmacokinetics exhibit relatively low bioavailability and relatively high variability compared to injections (Adjei et al., 1992). This can be ameliorated by the use of permeation enhancers (*vide infra*) which can enhance bioavailability and reduce variability (Hinchcliffe et al., 2005).

2.7. *Case examples of therapeutic areas sutiable for intranasal delivery*

The following sections provide case examples of therapeutic areas suitable for IN delivery. While the therapeutic areas are diverse, the common theme among them is an advantage for IN dosing, such as patient convenience and preference, rapid drug onset, avoidance of GI-related side-effects, and more consistent delivery for disease states associated with gastric dysmotility. These case examples range from products in exploratory development to marketed therapeutic products.

2.7.1. *Morphine for breakthrough cancer pain*

Patients with chronic cancer pain often manifest both incident and continuous pain. Incident pain, also described as “breakthrough pain”, is typical of rapid onset, is severe in intensity, and has an average duration of 30 min. Various researchers have reported on the investigation of IN morphine to treat this debilitating condition (Illum et al., 2002; Pavis et al., 2002; Fitzgibbon et al., 2003). Morphine has relatively low oral bioavailability due to extensive first-pass metabolism. Therefore, IN delivery provides an attractive option due to the avoidance of first-pass metabolism, non-invasiveness, and rapid onset of action. An example of human PK for IN, oral, and injection (IM) dosing of morphine is presented in Fig. 2. The data illustrate that IN dosing achieves a similarly fast drug onset ($T_{max} \sim 15$ min) compared with IM dosing, and is much faster than oral delivery ($T_{max} \sim 50$ min). As for any analgesic, speed of onset for IN morphine is highly desired for breakthrough cancer pain, since rapid onset of significant pain relief is critical.

2.7.2. *Treatments for migraine and cluster headaches*

Patients with recurrent migraine or cluster headaches may have difficulty managing their disease, and in extreme situations may require emergency room visits to control the pain. When compared with oral delivery, IN dosing provides very rapid drug onset, which is a critical factor for controlling headaches, as well as providing improved bioavailability. Similar to morphine for breakthrough cancer pain, IN analgesics for headache are most effective when the onset of action is rapid, and IN dosing provides a distinct advantage over oral dosing in this

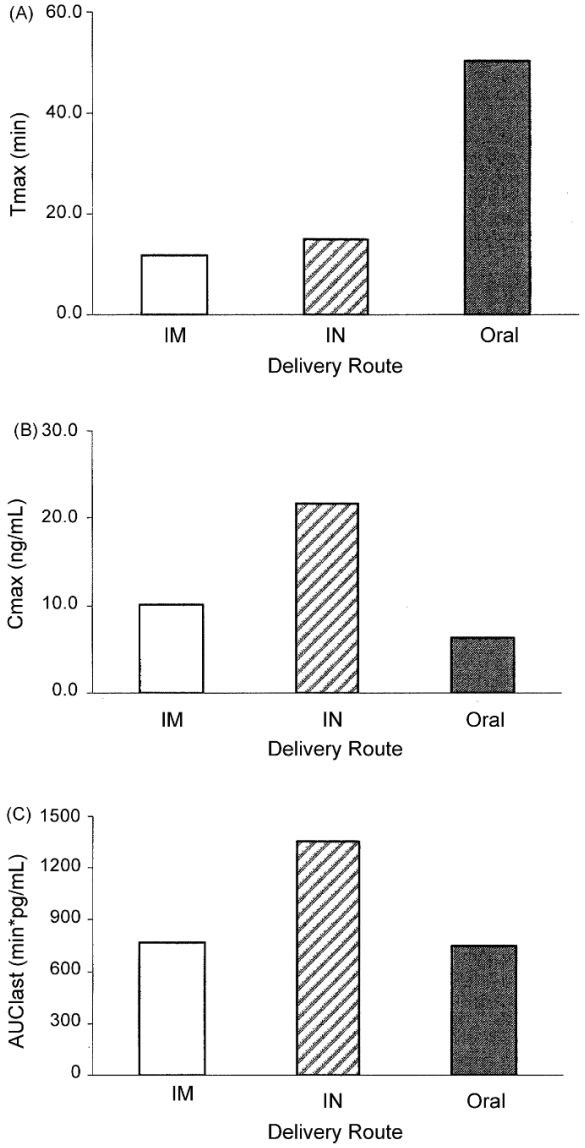

Fig. 2. PK parameters for morphine in humans: (A) T_{max} (min), (B) C_{max} (ng/mL) and (C) AUClast (min pg/mL). Data are shown for intramuscular (IM) dosing at 2.5 mg (white), intranasal (IN) dosing at 2.5 mg (striped) and oral dosing at 10 mg (grey). Data from Costantino et al. (2005).

regard. As an example, IN zolmitriptan for migraine treatment has been reported to provide significantly more rapid onset of therapeutic drug levels (Yates et al., 2002) and headache relief (Charlesworth et al., 2003) compared with oral dosing. Another important advantage of intranasal administration of drugs for treating migraines is that the therapeutic condition slows gastric emptying and hence oral drug absorption is compromised (Dahlof, 2002). Both oral and IN zolmitriptan are available commercially (under the trade name ZOMIG®). However, for this and other related applications, IN delivery provides a convenient and potentially more effective mode of dosing (Rapoport et al., 2004).

Butorphanol tartrate is another analgesic agent suitable for IN delivery. Butorphanol is extensively metabolized upon first-pass through the GI tract, and as a result, has very poor oral bioavailability (Gillis et al., 1995). The intravenous (IV) and intramuscular (IM) routes provide improved bioavailability and rapid drug onset, but at the cost of invasiveness, pain, and inconvenience. IN butorphanol offers a convenient alternative to IV and IM delivery and has been successfully developed commercially (marketed as STADOL NS®).

Other IN drugs have been explored for migraine and headache treatment (see Rapoport et al., 2004). Examples of drugs tested in humans include IN capsaicin for cluster headache treatment (Fusco et al., 1994), and migraine treatment using IN dihydroergotamine (Treves et al., 1998) and IN lidocaine (Maizels et al., 1996).

2.7.3. *Acetylcholinesterase inhibitors for Alzheimer's disease*

Kays Leonard et al. (2005) have reported on the development of IN galantamine, an acetylcholinesterase inhibitor indicated for the treatment of Alzheimer's disease. Pharmacokinetic testing revealed rapid drug onset for IN administration compared with conventional oral dosing. As with other drugs in its class, galantamine dosed orally has a clinically significant level of mechanism-based gastrointestinal (GI) side-effects such as nausea and vomiting. IN dosing dramatically reduced the emetic response, presumably as a result of avoidance of drug contact in the GI tract. Specifically, there was an order of magnitude reduction in emetic events (Fig. 3).

Patani et al. (2005) have explored an IN formulation of a heptylene-linked *bis*-tacrine analog (*bis*-THA). A series of investigations were conducted to examine various physicochemical properties (e.g., partition coefficient) of *bis*-THA compared with the parent molecule (tacrine). Permeation studies conducted using excised pig nasal mucosa revealed that the nasal mucosa was amenable for systemic delivery of *bis*-THA, and delipidization studies suggested that lipophilic components in the absorptive mucosa played a role in drug permeation.

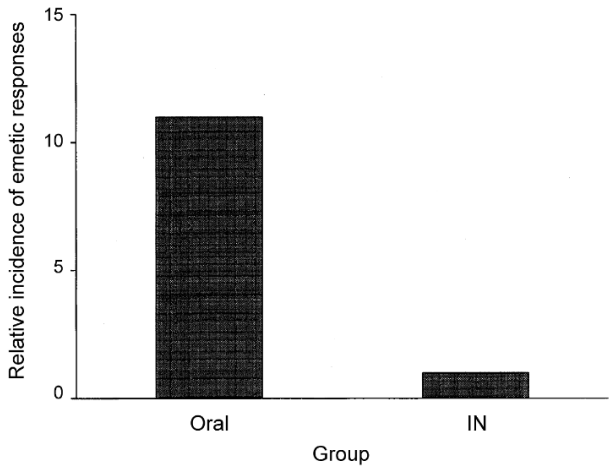

Fig. 3. Relative emetic response (in ferrets) for oral vs. IN dosing of galantamine. Oral dosing results in over a 10-fold increase in emetic responses. Data from Costantino et al. (2005).

2.7.4. Apomorphine

Apomorphine represents another example of a therapeutic agent in which various routes, including IN delivery, have been explored. Apomorphine is a dopamine receptor agonist with a high affinity for D_1 and D_2 receptor subtypes at sites within the brain known to be involved in the mediation of erection. It is currently approved for several indications, including an injection for the acute treatment of "off" episodes associated with Parkinson's disease (Johnston et al., 2005). Various in vivo studies have shown that the erectile effects of apomorphine are mediated at dopamine receptors in various nuclei of the hypothalamus and midbrain (Allard and Giuliano, 2001).

A case study describing IN apomorphine can be found elsewhere (Costantino et al., 2005). IN apomorphine is absorbed as rapidly as subcutaneous (SC) injection. The rapid onset for IN apomorphine is desirable for either erectile dysfunction or Parkinson's disease indications. Compared with sublingual (SL) dosing, IN delivery resulted in increased absorption, i.e., the bioavailability of SL apomorphine was only 56% that of IN apomorphine. Interestingly, the rates of significant adverse events were reduced dramatically after changing the route of administration to IN, even for a similar systemic exposure. For SL delivery, observed rates of nausea and vomiting were about 18–22% and 1–4%, respectively. In contrast, following IN delivery of a dose corresponding to about the same AUC as the SL dose, the incidence of nausea (3%) was nearly an order of magnitude less compared to sublingual delivery, and there were no incidences of vomiting.

2.7.5. Anti-nausea and motion sickness medications

Treatments for nausea and motion sickness represent additional therapeutic areas in which IN delivery provides advantages, including rapid onset and potentially more consistent dosing, compared to oral dosing due to issues with gastric dysmotility. IN metaclopramide has been explored for a variety of indications, including the prevention of postoperative nausea and vomiting (for a review see Ormrod and Goa, 1999). For IN dosing, both the absorption and elimination curves were similar to oral and IM administration (10 mg dose for all groups); it was concluded that oral and IN delivery were bioequivalent (Citron et al., 1987). In another related human PK comparison, Wenig (1988) reported similar C_{max} values for meclizine given by IN (10 mg), oral (10 mg) and IM (5 mg) routes. It was also reported that IN meclizine in rats and dogs provided similar PK to IV dosing and provided a more rapid onset and higher bioavailability compared with oral administration (Chovan et al., 1985).

Scopolamine, an antimuscarinic agent indicated for motion sickness, is another example of a drug in this area that is suitable for IN dosing (case study discussed by Costantino et al., 2005). Scopolamine has very low oral bioavailability due to extensive first-pass metabolism. Transdermal delivery provides an option, but this route of administration results in very slow onset and an unnecessarily prolonged effect, with significant side-effects including dry mouth, drowsiness, and blurred vision. Ahmed et al. (2000) reported on the human PK and side-effect profile of various IN scopolamine formulations. Compared with the transdermally delivered drug, IN scopolamine exhibited a more rapid onset. Although a variety of side-effects have been reported for transdermal scopolamine, no significant adverse effects were observed for the various IN formulations tested. Currently, IN scopolamine and IN promethazine are being explored for treating motion sickness in flight, including astronautical applications (Putcha, 2006).

Hussain et al. (2000) reported IN administration of the anti-emetic agent ondansetron in rats. IN ondansetron was completely absorbed; plasma concentration-time profiles for IN and IV were comparable. If these findings translate to human experience, IN ondansetron would provide a favorable alternative to oral dosing (absolute bioavailability of about 60%) (Roila and Del Favero, 1995).

2.7.6. Cardiovascular drugs

The feasibility of IN administration as an alternative route for administering cardiovascular drugs has been widely investigated (for a review see Landau et al., 1994). IN nitroglycerin has been explored clinically for controlling the hemodynamic response to orotracheal intubation (Grover et al., 1987; Hwang et al., 1995). Another example is provided by IN propanolol, a drug useful for immediate β-blockade prior to exercise in patients with angina. IN dosing of propanolol provides a PK profile in terms of rapidity and bioavailability that is very similar to IV administration, whereas oral dosing results in an order of magnitude decrease in bioavailability due to substantial first-pass metabolism (Hussain et al., 1980a,b). In a human clinical study, it was concluded that IN propranolol was effective in providing immediate β-blockade and improving exercise tolerance in patients with angina pectoris (Landau et al., 1993).

In addition, IN nifedipine was shown to be more suitable than oral delivery for perioperative blood pressure control (Kubota et al., 2001). In a similar human study by Kinoshita et al. (1990), it was discovered that IN nicardipine provided a more suitable hemodynamic effect than oral or tracheal delivery. A similar observation for the superiority of IN delivery when compared to oral delivery was reported for verapamil in dogs (Arnold et al., 1985), although this result has not been shown definitively in humans, likely the result of dose volume and drug concentration limitations (Watling et al., 1993).

2.7.7. Sedative agents (non-emergency situation)

A variety of reports have appeared in the literature regarding IN delivery of the benzodiazepine midazolam. IN midazolam has been investigated clinically for various applications, including treatment of panic disorder (Schweizer et al., 1992), and as a sedative to be given prior to, or in association with, a wide variety of surgical, dental, or other medical treatments in adults (Moss et al., 1993; Bjorkman et al., 1997) and children (Wilton et al., 1988; Rice and Kyff, 1990; Latson et al., 1991; Karl et al., 1992, 1993; Yealy et al., 1992; Hogberg et al., 1995; Theroux et al., 1993; Adrian, 1994; Hartgraves and Primosch, 1994; Harcke et al., 1995; Fishbein et al., 1997; Kogan et al., 2002). From various human studies, it has been observed that IN dosing results in more desirable pharmacokinetics and pharmacodynamics compared with other routes of administration such as oral and rectal; IN midazolam exhibits about 50–80% absolute bioavailability

and exhibits a very rapid onset (e.g., as fast as 5 min) (Rey et al., 1991; Walbergh et al., 1991; Kaufman et al., 1994; Bates et al., 1994; Fosel et al., 1995; Khazin et al., 1995; Malinovsky et al., 1995; Lejus et al., 1997; Fukuta et al., 1997; Geldner et al., 1997; Knoester et al., 2002). However, these studies also reported serious nasal irritation associated with IN midazolam (e.g., Kogan et al., 2002), which is a drawback to its use, particularly in children. Gudmundsdottir et al. (2001) have reported on a sulphobutylether-β-cyclodextrin formulation of IN midazolam that exhibited no serious adverse events in a human trial, but there were still reports of transient, mild to moderate irritation of nasal and pharyngeal mucosa.

A similar therapeutic in this class that has been explored for IN dosing is ketamine (Weksler et al., 1993; Louon and Reddy, 1994; Malinovsky et al., 1996; Diaz, 1997; Weber et al., 2003, 2004). Similarly to IN midazolam, IN ketamine has a pharmacokinetic and pharmacodynamic response equal or superior to that for oral or rectal administration. A study in anesthetized children demonstrated rapid onset of action similar to IV dosing, but with increased variability (Weber et al., 2004). Malinovsky et al. (1996) reported that the absolute bioavailability of IN ketamine was about 50% with a maximal plasma concentration (C_{max}) achieved at about 20 min, whereas rectal ketamine provided only about half the absolute bioavailability (25%) and required twice as much time to attain an equivalent C_{max} (about 40 min). Another potential application of IN ketamine is the control of breakthrough pain for patients with chronic pain (Carr et al., 2004).

It should be noted that IN opiates also have been explored for achieving sedation. Opiates evaluated for this indication include IN sufentanil (Vercauteren et al., 1988; Henderson et al., 1988; Helmers et al., 1989; Zedie et al., 1996) and morphine congeners (Skopp et al., 1997).

2.7.8. Examples for application in an emergency situation

IN delivery is an attractive option for the delivery of drugs in an emergency setting (Wolfe and Bernstone, 2004). In emergency situations, the ease of dosing and rapidity of pharmacokinetic response is essential. Nasal administration of drugs in this setting can meet these needs, presuming a relatively simple device is chosen (e.g., nasal spray bottle). IN delivery is relatively simple and convenient, therefore allowing a nurse or even the patient to administer an IN product, as opposed to requiring a physician. The following are brief examples illustrating the potential niche for IN administration to address the unmet medical needs of numerous emergency care situations.

Naloxone is used for the treatment of opioid overdose, and is typically administered by injection, e.g., NARCAN®. IN dosing of naloxone has been explored in emergency settings for human dosing and could provide significant advantages to both patient and caregiver (Loimer et al., 1994; Barton et al., 2002). IN naloxone provides a PK profile similar to IV dosing in terms of bioavailability and onset of drug levels (Kendall and Latter, 2003). Additionally, IN naloxone can be administered rapidly, which could be important due to the delay in therapy associated with establishing an IV access in an IV drug abuser.

Children suffering intractable seizures offer another example in which IN delivery may be advantageous in an emergency setting. IN benzodiazepines (O'Regan et al., 1996; Kendall et al., 1997; Wallace, 1997; Fisgin et al., 2000) have been administered for this purpose, and also have utility for sedation in a non-emergency setting, as described above. It can be very difficult to establish an IV access in children suffering from intractable seizures, therefore, alternate methods of administering antiepileptics (e.g., rectal) are necessary. Compared with rectal administration, IN delivery has been shown to be superior in terms of efficacy (Fisgin et al., 2002), as well as patient and caregiver preference (Wilson et al., 2004).

Glucagon is a case in which IN dosing has been tested in humans as an alternative to injections (Freychet et al., 1988; Rosenfalck et al., 1992; Pontiroli et al., 1993; Pontiroli, 1998; Stenninger and Aman, 1993; Hvidberg et al., 1994). The IN form of this drug, used for treating insulin-induced hypoglycaemia, provided rapid drug onset. It can be easily administered by a family member, and is safer and faster than oral glucose in an unconscious patient.

2.7.9. Systemic delivery of macromolecules

Peptides and proteins represent an interesting opportunity for IN administration (Pontiroli, 1998). A representative listing of various clinical and preclinical studies of IN peptides and proteins (in the absence of permeation enhancers) is presented in Table 1. As discussed in more detail below, the relatively high-molecular-weight and fragility of such therapeutics make delivery problematic. Injection represents the standard delivery route, albeit an invasive one. Of the hundreds of macromolecular products developed (Costantino, 2004), only a handful of non-injection products have reached the market, including two pulmonary products (i.e., Pulmozyme® and Exubera®) and several IN peptide formulations. Examples of marketed IN peptide products include salmon calcitonin (e.g., Miacalcin®), desmopressin (e.g., DDAVP), and nafarelin (e.g., Synarel®).

IN delivery represents a clinically advanced non-injection option for the delivery of high-molecular-weight drugs. However, in the case of these marketed IN peptide products, the formulations employed are typically devoid of permeation enhancers, and as the molecular weight is relatively high, IN bioavailability is relatively (and expectedly) low. For example, it is reported that the bioavailability of IN salmon calcitonin (MW of about 3432 Da) is about 3% (Novartis Pharmaceuticals, 2006).

The bioavailability of IN macromolecules can be dramatically improved by using permeation enhancers (see Table 2 for a representative listing). A thorough treatment of this area is outside the scope of the current review. Examples of excipients shown to improve nasal permeation include bile salts (Moses et al., 1983; Aungst and Rogers, 1988; Hosoya et al., 1999; Bagger et al., 2001), alkyl glycosides (Ahsan et al., 2001; Pillion et al., 2002; Nakamura et al., 2002; Mustafa et al., 2004), polymers (e.g., poly-L-arginine (Ohtake et al., 2002), gelatin (Wang et al., 2002), and chitosan (Illum et al., 1994; Prego et al., 2005)), tight junction modulating peptides (Johnson and Quay, 2005; Chen et al., 2006), lipids and surfactants (Coates et al., 1995; Laursen

Table 1
Examples of bioavailability for various peptides and proteins administered IN in the absence of permeation enhancers

Peptide/protein	Drug MW (Da)	Bioavailability (%)[a]	Reference
ACTH 4-9	906	10% (rabbit); 15% (rat)	Schipper et al. (1993)
ACTH 4-10	962	7.6%	Bickel et al. (1988)
Octreotide	991	18% vs. SC (human)	Kissel et al. (1992)
Desmopressin aceate	1,183	9–20% (human)	Harris et al. (1988)
Leuprolide acetate	1,209	2.4% (human)	Adjei et al. (1992)
Buserelin	1,239	6% (human)	Holland et al. (1986)
Nafarelin	1,321	2.8% (human)	Chaplin (1992)
GHRH (1-29)	3,358	3–5% (human)	Wilton et al. (1993)
Salmon calcitonin	3,432	3% vs. IM (human)	Novartis Pharmaceuticals (2006)
$PTH_{1–34}$	4,118	2%[b] (human)	Matsumoto et al. (2006)
Insulin	5,808	0.9% (rabbit); 0.3% (rat)	Deurloo et al. (1989)
Hirudin-2	6,900	2.14% vs. SC (rat)	Zhang et al. (2005)
rhG-CSF	∼18,800	2% (rat)	Machida et al. (1993)
Interferon-α B/D	∼19,000	2.9% (rabbit)	Bayley et al. (1995)

Abbreviations used: ACTH, adrenocorticotropic hormone; GHRH, growth hormone releasing hormone; IM, intramuscular; PTH, parathyroid hormone; rhG-CSF, recombinant human granulocyte-colony stimulating factor; SC, subcutaneous.
[a] Absolute bioavailability (compared to IV dosing), unless otherwise noted.
[b] Based on pharmacodynamics.

et al., 1996; Mitra et al., 2000), cyclodextrins (Merkus et al., 1991; Schipper et al., 1993; Matsubara et al., 1995; Marttin et al., 1997), and chelators (Hosoya et al., 1994). Enhancers used in chronically administered nasal products should exhibit low toxicity.

3. Drug characteristics

Before selecting the route of administration for a drug, it is necessary to consider in detail: (i) the therapeutic purpose of the drug, (ii) the physicochemical characteristics of the drug itself, and (iii) the delivery system necessary to deliver the drug safely and efficiently to the target. When considering the therapeutic purpose of a drug, the nasal route of administration is a suitable choice for drugs in which a rapid onset of action is needed (e.g., in the treatment of acute pain, nausea, erectile dysfunction, or Parkinson's disease). Furthermore, the nasal route may be a preferred alternative to other routes of administration for diseases that need multiple dosing, or for drugs where other routes of delivery are difficult to use, such as for peptides and proteins, or for vaccines.

Drug permeation via the nasal route is influenced by a variety of structural, biochemical, and physiological properties of the nasal mucosa, as well as by the physicochemical characteristics of the drug and its formulation (Arora et al., 2002). Depending on their lipophilicity, drugs permeate nasal tissue transcellularly (characteristic for hydrophobic, low-molecular-weight therapeutics) or paracellularly (typical for more hydrophilic drugs or in the case where tight junctions are opened). Physical characteristics of the drugs such as molecular weight, lipophilicity, stability, solubility, and dose are highly important not only for the choice of the route of delivery, but also for the selection or development of an appropriate delivery system. The importance of these physicochemical characteristics and the considerations for the choice of formulation approaches for the nasal route of delivery will be discussed first.

3.1. Physicochemical characteristics

When drugs are administered to the nasal cavity (in the form of a nasal spray or a nasal powder) for systemic effect, the drug meets a number of barriers, including the mucous layer, the

Table 2
Examples of bioavailability for various peptides and proteins administered IN in the presence of permeation enhancers

Peptide/protein	Drug MW (Da)	Bioavailability (%)[a]	Reference
ACTH 4-9	906	17% (rabbits); 65% (rats)	Schipper et al. (1993)
Salmon calcitonin	3,432	27% (rat)	Matsuyama et al. (2006)
$PYY_{3–36}$	4,050	16% (human); 19% (rabbits)	Park et al. (2004)
$PTH_{1–34}$	4,118	5–8%, 12–15% vs. SC (human)	Brandt et al. (2006)
Insulin	5,808	3–13% (human, various studies)	Hinchcliffe and Illum (1999) and references therein
rhG-CSF	∼18,800	8.4% vs. SC (sheep)	Gill et al. (1998)
Human growth hormone	∼22,000	3.8–8.9%	Laursen et al. (1996)
Interferon-β	∼22,500	19–21%[b] vs. IM (human)	Vitkun et al. (2004)

Abbreviations used: ACTH, adrenocorticotropic hormone; GHRH, growth hormone releasing hormone; rhG-CSF, recombinant human granulocyte-colony stimulating factor.
[a] Absolute bioavailability (compared to IV dosing), unless otherwise noted.
[b] Based on pharmacodynamics.

epithelial membrane, and associated junctional barriers. A drug will need to penetrate or overcome these barriers in order to reach the capillaries below the mucosal layer. Some researchers (Kearney and Marriott, 1987; Henry et al., 1992) have shown that in the gastrointestinal tract (where the mucus has a thickness of about 500 μm) the rate of absorption of drugs is impaired by the mucous layer. The nasal mucus is only a few microns thick, therefore it may not present a substantial diffusional barrier. However, more work in this area is warranted.

The diffusion of compounds with various physicochemical characteristics in mucus was measured in a study completed by Larhed et al. (1997). For small molecules, the most important factor governing diffusion was compound lipophilicity. For large molecular weight drugs, such as peptides, intermolecular interactions (e.g., hydrogen bonding and ionic interactions) between the peptide and the glycoprotein chains of the mucus could to some extent constitute a barrier. Larhed et al. (1997) demonstrated that higher molecular weight was associated with slower diffusion through gastrointestinal mucus. In another study, it was also shown that mucolytic agents that decrease the viscosity of the mucus can improve nasal drug absorption for some peptides (O'Hagan et al., 1990).

Following passage through the mucous layer, the drug must traverse the epithelial membrane transcellularly or the junctional intracellular barriers paracellularly. Lipophilic drugs are able to partition into the lipid environment (bilayer) of the cell membrane and diffuse into and transverse the cell in the cytoplasm. Such drugs therefore pass directly through the cells (transcellular pathway) by a process of passive diffusion. Active processes involving receptor mediated or vesicular transport mechanisms could be of relevance to certain compounds (McMartin et al., 1987).

3.1.1. Molecular weight

In the absence of permeation enhancers, nasal absorption drops off sharply for drugs with a molecular weight over 1000 Da (McMartin et al., 1987; Fisher et al., 1987). For water-soluble compounds, there is a linear correlation between the log(%drug absorbed) and the log(molecular weight). With the use of enhancers, the intranasal bioavailability of peptides and even small proteins can be improved.

Many lipophilic drugs administered nasally are of low-molecular-weight (<1000 Da), and the transport across the mucosal membrane is relatively efficient compared to larger lipophilic drugs. For polar drugs that are transported via the paracellular route, molecular weight is a very important determinant of the rate and degree of transport. Nasal epithelial cells are inter-connected on the apical side by a series of narrow belt-like structures called the junctional complexes, including tight junctions (TJs), which form a dynamic, regulatable, semi-permeable diffusion barrier between the epithelial cells.

This TJ diffusional barrier allows the interchange of small ions between the apical and the basolateral side of the cell membrane; however, the transport of larger molecules is limited. For example, there is limited transport of molecules larger than 3.6 Å, and for molecules larger than 15 Å, the transport is negligible (Stevenson et al., 1988; Madara, 2000). Not surprisingly, electron microscopy data has been reported that visualizes the TJs as a network of strands that appear as rows of 10 nm particles within the plane of the plasma membranes of neighboring cells (Staehelin, 1973; Anderson, 2001). It is hypothesized that these strands contain pores that dynamically open and close (Claude, 1978).

It is enlightening to compare the dimensions of the TJs with that of some model peptides and proteins. For globular peptides and proteins, molecular dimensions can be roughly estimated using an average partial specific volume of 0.73 cm^3/g (Harpaz et al., 1994). Using this approach for the model protein lysozyme (MW = 14,320) yields a calculated diameter of about 34 Å compared with the measured dimensions of approximately 30 Å × 30 Å × 45 Å (Imoto et al., 1972). In the case of very small peptides, for instance octreotide (MW = 991 Da), the estimated diameter is on the order of 10 Å.

The shape of many peptides and proteins can be approximated as a sphere in solution; deviation from sphericity can be expressed as the frictional ratio f/f_0 above unity, where f_0 is the rate of diffusion of a molecule of the same size but of true spherical shape. Typical globular proteins have frictional ratios in the range of 1.05–1.38 (Florence and Attwood, 1998). Due to their size and shape factors, peptides and proteins would be expected to have a very low degree of absorption from the nasal cavity via tight junctions.

As described later in this review, a range of signal transduction processes (e.g., protein kinase C) that can activate the opening and the closing of the tight junctions regulate the barrier properties and the assembly of the tight junctions (Smith et al., 2005). Such processes may be affected by the use of certain nasal "tight junction" absorption enhancers, potentially improving the passage of molecules through the tight junctions (Illum, 2005; Johnson and Quay, 2005).

Many research groups have examined the effect of molecular size on absorption across tissue barriers using a variety of mucosal cell types including the respiratory (Schanker and Hemberger, 1983) and the gastrointestinal (Loehry et al., 1973) tracts. This relationship is well researched (Gennaro, 2000). Fisher et al. (1987) were the first to investigate the effect of molecular weight on nasal absorption by administering a range of water-soluble model compounds (i.e., 4-oxo-4H-1-benzopyran-2-carboxylic acid, *para*-aminohippuric acid, inulin, and dextran) (MW range of 200–70,000 Da) to rats using a modified "Hirai model". It was concluded for water-soluble hydrophilic compounds that an inverse relationship existed between the proportion of the dose absorbed and the molecular weight. For the lowest molecular weight, 100% absorption was achieved as compared to about 15.5% for 5200 Da and 2.3% for 70,000 Da. It should be mentioned that in the "Hirai model" the mucociliary clearance mechanism is impaired, resulting in higher nasal absorption than would be anticipated in man (Major and Illum, 1997).

Later studies in rats (Fisher et al., 1992) further investigated the relationship between molecular weight and nasal absorption using fractionated labeled dextrans (range in MW of 1269–45,500 Da). The same trend was examined in rabbits (Hosoya et al., 1993) as a comparative study utilizing the trans-

port of FITC-dextrans (4400–71,200 Da) to assess differences in the structural barrier functions of excised nasal, buccal, duodenal, jejunal, ileal, and rectal tissue. It was observed that of all the tissues evaluated, the nasal tissue was the most permeable. Later, a similar study was carried out in vivo in rats on a range of water-soluble model compounds (i.e., phenol red, tryptan blue, and FITC-dextran, with molecular weights from 354 to 9100 Da). These compounds were administered via the lungs, nose, buccal cavity, and the small and large intestine, in order to compare the extent of absorption and the effect of molecular weight from each site (Yamamoto et al., 2001). These authors found the lungs to have the highest absorption, followed by the nasal cavity, which was similar to the small intestine. The buccal cavity showed the lowest absorption capacity after the large intestine. Both studies confirmed the effect of molecular weight on absorption.

McMartin et al. (1987) evaluated the structural requirements for the absorption of drugs across the nasal cavity by combining absorption data from about 30 drugs. They confirmed the results found by Fisher et al. (1987) and anticipated that efficient absorption should be expected for a molecular weight of less than 1000 Da, whereas larger molecular weight proteins, such as horseradish peroxidase (HRP) (34,000 Da) would only give 0.6% bioavailability. It was speculated that such large proteins were transported via the transcellular route possibly in vesicles or by receptor mediated transport. Interestingly, Masuda (1985) used electron microscopy to demonstrate this type of nasal transport of HRP in guinea pigs.

The inverse relationship between drug molecular weight and its permeation rate has been observed for nasal formulations containing permeation enhancers. This was demonstrated by Donovan et al. (1990), who evaluated in rats the IN delivery of polyethylene glycol (PEG) ranging from 600 to 2000 Da in the absence and presence of three absorption enhancers: sodium glycocholate, sodium lauryl sulphate, and polyoxyethylene-9-lauryl ether. Although each absorption enhancer increased the absorption of PEG in a concentration-dependent manner, the trend of PEG molecular weight on absorption was unaffected. Marttin et al. (1997) evaluated the nasal absorption in rats of FITC-labeled dextrans (3000–10,000 Da) with and without methylated β-cyclodextrin (β-CD) or sodium taurodihydrofusidate (STDHF) as an enhancer. They also found that the addition of an enhancer with a known profound effect on the cell membrane (STDHF), as compared with one positioned mainly to affect the tight junctions (β-CD), did not alter the route of transport of these hydrophilic compounds (which remained paracellular). Miyamoto et al. (2001) studied the effect of poly-L-arginine, a specific tight junction absorption enhancer, on the nasal absorption in rats of a range of FITC-labeled dextrans (4300–167,000 Da). The results were consistent with the studies above, namely that the bioavailability of the dextrans appeared to decrease exponentially with increasing molecular weight, both with and without the added enhancer.

For the direct transport of drugs from the nasal cavity to the CNS, the drug is proposed to pass the olfactory epithelial barrier rather than the respiratory epithelium, using one of two different transport mechanisms as described by Illum (2000). Sakane et al. (1995) discovered that with nasal administration of labeled dextran (4400–40,500 Da) to rats, the appearance of the dextran in the cerebral spinal fluid (CSF) decreased with increasing molecular weight. The level of CSF uptake for 20,000 Da dextran was about a third of that observed with the lowest molecular weight dextran, and the quantities transported to the CSF were small (0.01–0.1%). The mechanism of transport was most likely paracellular, due to the rapid appearance of the dextran in the CSF.

3.1.2. Hydrophobicity/hydrophilicity

Absorption of compounds that cross biological membranes and mucosal barriers, such as the gastrointestinal tract, may be affected by the hydrophobic/hydrophilic balance of the compound, and for weak acids or bases, by the pH of the environment ("the pH partition theory"). For nasal mucosal membranes, a range of studies evaluating the effect of lipophilicity and pH on nasal absorption of small molecular weight drugs has been performed in the rat model. Very early on, Hirai et al. (1981a) studied the absorption of salicylic acid and aminopyrine from the nasal cavity at a range of pH values and found that the largest absorption of the drugs occurred when they were in their non-ionized state, in which drugs have a higher apparent partition coefficient, i.e., are more lipophilic. However, significant absorption was also seen when the drugs were ionized.

Hussain et al. (1985) performed a similar study evaluating the effect of pH values from 2 to 7.1 on the absorption of benzoic acid during 60-min nasal perfusion. The drug was absorbed to the highest degree in its non-ionized form (pH 2.5, 44%), but even at 99.9% ionization (pH 7.19), 13% absorption was observed. The rate of absorption was calculated to be 4 times faster for the non-ionized species than for the ionized.

A later study by Gibson and Olanoff (1987) found the nasal transport of decanoic, octanoic, and hexanoic acids to be greatest at pH 4.5, which is near the pK_a values at which the compounds are ~50% ionized. A likely explanation is that these acids may have poor water solubility below this pH value. The study also showed that the transport of a series of steroids was directly related to their lipophilicity, and as expected, was pH independent. Corbo et al. (1989) presented similar results on the nasal absorption of progesterone and its derivatives, which showed increased nasal absorption of progesterone compounds with increasing lipophilicity. It is interesting to note that in a study regarding the transport of a range of barbiturates (Huang et al., 1985a), a 40-fold increase in the partition coefficient gave rise to only a 4-fold difference in absorption, even though the rate of transport was dependent on the lipophilicity of the molecule. It was also discovered that acyl esterification of L-tyrosine resulted in a higher partition coefficient than for L-tyrosine, and that this group of compounds was absorbed across the nasal tissue with a faster rate than L-tyrosine, whereas *N*-acetyl-L-tyrosine esters had partition coefficients and absorption rates similar to the parent compound. The difference in absorption rate was attributed to the absence of the negative charge on the carboxylate moiety of the molecule rather than the higher lipophilicity (Huang et al., 1985b). However, it has recently

been shown by Yang and Mitra (2001) that the nasal absorption of L-tyrosine conjugates probably is through an amino acid carrier-mediated pathway, which would be dependent on the concentration but independent of the partition coefficient of the molecule.

Shao et al. (1994) synthesized and administered a series of acyclovir prodrugs to the nasal cavity of rats. Absorption increased with increasing lipophilicity and the parent acyclovir (partition coefficient ~0.018) showed no apparent absorption. Kimura et al. (1991) evaluated the nasal absorption of a series of quaternary ammonium compounds with increasing molecular weight and increasing lipophilicity. In these studies, nasal absorption was shown to be highest for the lowest molecular weight compound with the lowest lipophilicity and lowest for the highest molecular weight compound and the highest lipophilicity. This is not surprising since these types of molecules are ionized at all pH values. It is likely that the compounds were crossing the membrane via the paracellular route, for which size is more important than lipophilicity.

Sakane et al. (1991) utilized a rat nasal perfusion model to evaluate the influence of drug lipophilicity on transport from the nasal cavity to the CNS. Four different sulpha drugs (sulphanilic acid (SA), sulphamethizole (SMZ), sulphisoxazole (SIX), and sulphisomidine (SID)) with increasing isoamyl alcohol/phosphate buffer partition coefficient (0.012, 0.250, 0.261 and 0.892, respectively) were administered nasally, and the drug concentration in the CSF measured. There was a linear increase in CSF concentration with increase in drug lipophilicity. For SMZ, the quoted partition coefficient corresponded to an octanol/water partition coefficient of 0.540. Hence, the range of lipophilicities is at the lower end of the scale for drug compounds. It would be expected that the amount of drug reaching the CSF would decline due to a higher uptake from the nasal cavity directly to the bloodstream. In contrast, Chou and Donovan (1997) found that there was no distinct correlation between the log distribution coefficient (log DC) and the CSF uptake when investigating the transport from the nasal cavity to the CSF for a range of antihistamines with log distribution coefficients (chloroform/buffer) between 0.35 and 2.91. The drugs all had similar pK_a values and were ionized (>90%) at the buffer pH (6.8). It was concluded that the transport of these drugs, both to the blood and the CSF, seemed to be controlled by a combination of molecular and physiological properties, none of which singularly determined the tissue distribution. On the other hand, Kao et al. (2000) showed that after nasal administration of two alkyl ester prodrugs of L-dopa, the more lipophilic prodrug (butyl ester, partition coefficient ~7.17) resulted in higher L-dopa levels in both the CSF and in the olfactory bulb than the methyl ester (partition coefficient ~0.25, thereby supporting the results obtained by Sakane et al. (1991).

Sakane et al. (1994) later investigated the relationship between the degree of dissociation of sulphisomidine (pK_a 7.4) and its uptake into the CSF. The drug was perfused in the nasal cavity of rats at pH values from 5.5 to 9.4. It was shown that both the systemic absorption and the CSF concentration of the drug decreased with the degree of ionization and was highest at basic pH values.

3.1.3. Chemical and physical stability

Many drugs are susceptible to some form of chemical or physical instability such as degradation by hydrolysis or oxidation, isomerizsation, photochemical decomposition, or polymerization. These processes can lead to loss of drug potency or change in the physical appearance of the formulation such as discoloration (e.g., due to the degradation product possessing a different color). In general, peptides and protein drugs have greater fragility compared to low-molecular-weight pharmaceuticals. Rational formulation strategies can be employed in order to prevent or limit such deleterious processes during the storage of formulations. It is beyond the scope of this review to discuss in detail these various formulation approaches. Several case examples will serve the purpose of illustration.

As a representative case, apomorphine is an IN drug candidate that is susceptible to oxidation of the hydroxyl groups of the catechol moiety to the quinone form in aqueous solution (Subramony, 2006). This oxidation causes a color change and poses a delivery challenge. One successful approach to mitigate degradation in the aqueous IN formulation is the addition of appropriate antioxidant systems (Achari et al., 2004). A potential alternate approach is to deliver the drug in the powder form; reaction rates are typically lower in the solid compared with the aqueous state (Merkus, 1998).

Diamorphine, a related low-molecular-weight compound, is very unstable in solution due to its degradation to 6-monoacetylmorphine and then to morphine (Hutchinson and Somogyi, 2002). As an injectable formulation, it is available as a lyophilized product for dissolution with a 5% dextrose solution before injection. Such formulation concepts can be adapted to nasal delivery, in which a powder system is reconstituted in a suitable vehicle and delivered in solution form (albeit at the expense of a potentially more complicated dosage preparation for the patient). Alternatively, a powder formulation can be directly administered. Kendall and Latter (2003) reported on the nasal administration of a diamorphine dry powder formulation to patients. The powder formulation would be expected to dissolve rapidly once in the nasal cavity. The pharmacodynamic effects of a nasally administered powder formulation of diamorphine were very similar to those seen for an intramuscular injection, with a more rapid onset of action.

Finally, it should be noted that peptides and proteins tend to be relatively fragile (for a review, see Stotz et al., 2004), necessitating rational stabilization for their intranasal formulations. To this point, multiple intranasal peptide products that achieve a suitable shelf life have been developed (even though refrigeration may be necessary until the product is used by the patient, e.g., Miacalcin®).

3.1.4. Biochemical stability

The environment within the nasal cavity has the ability to metabolize xenobiotics by a defensive enzymatic barrier mechanism. For example, many compounds undergo metabolism via the enzyme cytochrome P450, which is present in the nasal mucosa in quantities second only to those present in the liver (when expressed per gram tissue). Cytochrome P450 belongs to a multigenic family of enzymes displaying broad substrate

specificity and catalyzing a NADPH-dependent monoxygenation of lipophilic xenobiotics or endogenous substrates (Minn et al., 2002). Compounds such as *N*-nitroso compounds (Brittebo and Tjalve, 1981), phenacetin (Brittebo, 1987), progesterone (Brittebo, 1982), estradiol (Brittebo, 1985), and testosterone (Lupo et al., 1986) have been shown to undergo some degree of metabolism in the nasal cavity via this mechanism. Due to the increasing interest in nose-to-brain delivery of drugs, recent focus also has turned toward the metabolic barrier of the olfactory epithelium. As with the respiratory epithelium, it has been shown that the olfactory epithelium contains a high activity of P450 (Minn et al., 2002).

Yang et al. (2001) studied the enzymatic metabolism of acyclovir by a nasal perfusion technique in the rat. Prodrugs in the form of esters of acyclovir were synthesized in order to evaluate their ability to increase nasal absorption by decreasing the nasal degradation of the compound. An L-aspartase β-ester was found to be the most stable compound of those studied, leading to an increase in the bioavailability of the drug. It should be emphasized that the animal procedure used involves perfusion of the drug through the nasal cavity for 90 min, and as such would probably over-estimate the degradation that could take place in vivo.

The low bioavailability of most peptides and proteins after nasal delivery can be attributed to their relatively large molecular weight as well as proteolysis in the nasal cavity (O'Hagan et al., 1990). This aspect has been reviewed in detail by several researchers (Lee, 1988; Wearley, 1991; Sarkar, 1992; Harris, 1993). Enzymes present within the lumen of the nasal cavity and in the tissue comprise exopeptidases, such as mono- and diaminopeptidases, and endopeptidases such as serine, cysteine, and aspartic proteinases. These enzymes are able to cleave peptides and proteins at their N and C termini and at an internal peptide bond, respectively. However, peptides and proteins are susceptible to attack at more than one site at a given time. Most studies on the degradation of peptides have been carried out using tissue homogenates, resulting in a potential overestimation in the degree of degradation (Hirai et al., 1981b; Kashi and Lee, 1986). The latter researchers found that various enkephalins were all rapidly degraded, but that the hydrolysis rates could be halved in the presence of the enzyme inhibitor bestatin. Jonsson et al. (1992) found that a rabbit nasal mucosa homogenate rapidly degraded desmopressin in vitro. Lee (1988) has commented that almost half of the aminopeptidase activity in the nasal cavity is membrane bound, whereas for other mucosal tissues it is found in the cytosol.

A number of reports have described improved bioavailability of peptide drugs in the presence of agents acting as local peptidase/protease inhibitors on the nasal mucosa. For instance, enzymatic activity can be reduced by the addition of enzyme inhibitors, including bestatin, amastatin, boroleucin, borovaline, aprotinin, and trypsin inhibitor (Suda et al., 1976; Hussain et al., 1989; Hoang et al., 2002), and also by certain absorption enhancers that have enzymatic inhibition activity, e.g., bile salt and fusidic acid (Hanson et al., 1986). Recently, the analgesic effect of leucine enkephalin (Leu-Enk) and its synthetic analogue [D-ala(2)]-leucine enkephalinamide (YAGFL), with and without enzyme inhibitors and/or absorption enhancers, was investigated using the acetic acid-induced writhing test in mice (Gwak et al., 2003). The addition of enzyme inhibitors and absorption enhancers markedly increased the analgesic effect of the two drugs in the animal model. The enzyme inhibitors that gave the best effects were azelaic acid (1%) and thimerosal (0.5 mM). Similarly, Agu et al. (2004) found a remarkable increase (4 to 94-fold) in the permeation of methionine enkephalin across excised human nasal tissue when administered in combination with bestatin or puromycin as enzyme inhibitors and glycocholate and dimethyl-β-cyclodextrin as absorption enhancers.

Various groups have attempted to increase the nasal bioavailability of different calcitonins by decreasing the vulnerability of this peptide to enzyme degradation. Hence, Watanabe et al. (1998) administered elcatonin (an eel calcitonin derivative where the S–S bond is replaced by the stable C–N bond) to the nasal cavity in rabbits and evaluated the effect of the addition of a protease inhibitor, nafamostat mesylate, on the absorption of the drug. They found that elcatonin was efficiently absorbed nasally and that the enzyme inhibitor was able to significantly enhance absorption. The addition of an endocytotic inhibitor did not change the uptake. Another group produced PEGylated salmon calcitonin, which showed strong resistance against enzymatic degradation in the rat nasal mucosa (Na et al., 2004). This PEGylated salmon calcitonin showed higher bioavailability after nasal administration to rats. Such strategies to increase the stability of a molecule will result in the creation of a new chemical entity, resulting in a need for toxicological studies.

In another example, Kleppe et al. (2006) have explored IN glucagon-like peptide 1 (GLP-1) for type II diabetes. This molecule has a short half-life in vivo as a result of degradation by dipeptidyl peptidase-IV (DPP-IV), necessitating multiple dosings per day. For such a regimen, IN dosing may be favored by the needle-naïve patient population compared to frequent injections. A formulation with permeation enhancers that exhibits low cytotoxicity, good cell viability, and high permeation has been identified. The PK of IN GLP-1, with and without a DPP-IV inhibitor, was tested in rabbits. Compared with permeation enhancers alone, the inclusion of the DPP-IV inhibitor increased the absolute bioavailability of GLP-1 over 5-fold (see Fig. 4).

3.1.5. Solubility

Typically, it is common that intranasal drugs are administered to the nasal mucosa as a molecularly dispersed form, e.g., in solution. The allowable volume of solution for IN administration is relatively low, and therefore drugs with low aqueous solubility and/or requiring high doses may present a problem. When a drug is administered as a powder formulation to the nasal cavity, a dissolution process will precede the absorption process. Dyer et al. (2002) described such a system in the form of chitosan powder containing insulin. The drug dissolves in the fluid present in the nasal cavity.

Strategies based on non-dissolving particulate systems for nasal delivery, such as nanoparticles, have been reported recently in the literature. It is believed that nanoparticles (and perhaps even microparticles) can be transported across the nasal cavity

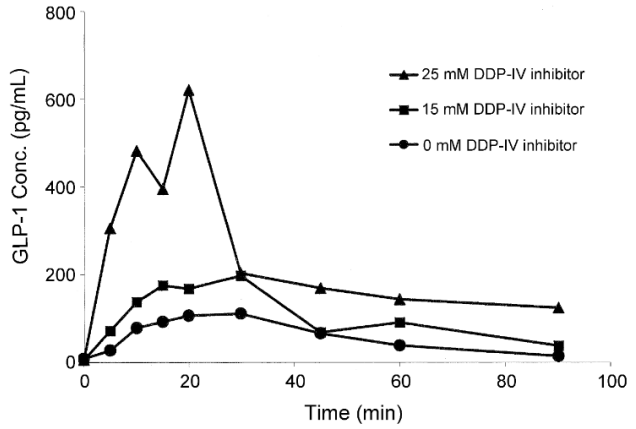

Fig. 4. PK of IN GLP-1 with permeation enhancers in absence (filled circles) and presence at 15 (filled squares) and 25 (filled triangles) mg/mL DDP-IV inhibitor. Compared to enhancers alone, the inclusion of the DPP-IV inhibitor resulted in up to a 5-fold increase in bioavailability. Data from Kleppe et al. (2006).

into the bloodstream without prior dissolution. This has been shown for polystyrene model particles of sizes 20–1000 nm (Brooking et al., 2001). The uptake was size dependent (e.g., the smaller the size the higher the uptake), as well as highly dependent on the surface characteristics of the particles. The mechanism of transport was suggested to be an uptake into the nasal-associated lymphoid tissue, in particular into the cells similar to the M-cells in the gut.

Due to the anatomy and size of the nasal cavity, only a relatively low volume of a liquid formulation can be administered, i.e., about 100–150 μL in each nostril. Although some exceptions exist (e.g., FluMist® is ~1 mL volume), these cases may represent a substantial amount of the drug dose being swallowed. Therefore, it is sometimes necessary to increase the solubility of the drug in order to allow for delivery of a therapeutically relevant dose. There are several approaches that may increase the solubility of poorly soluble compounds for nasal administration, some of which will be discussed here: (i) the use of pro-drugs; (ii) addition of co-solvents, (iii) use of cyclodextrins as solubilizing excipients, and (iv) choice of salt form.

3.1.5.1. Prodrugs. Often the prodrug principle is applied in an effort to modify the properties of a drug so as to improve its lipophilic character, ultimately increasing its transport across a biological membrane. An example is the conversion of adrenaline to the prodrug dipivoyl adrenaline for enhanced penetration of the corneal barrier (Florence and Attwood, 1998). The more lipophilic pro-drug was absorbed to a greater extent than the parent drug and was then hydrolyzed to the active compound adrenaline in the aqueous humor.

Some researchers have also used the prodrug approach for improving the solubility of poorly soluble drugs. For example, the drug L-dopa has a low water solubility of 1.65 mg/mL, which creates a problem for the nasal administration of an effective dose of 10 mg in a volume of 125 μL. Kao et al. (2000) have described the production of L-dopa prodrugs in the form of various alkyl esters. For the butyl ester prodrug, the solubility was increased to 660 mg/mL, which made it possible to produce an aqueous nasal formulation of a suitable concentration. Once in the blood stream, the prodrug was converted rapidly to the parent drug.

In another example of this formulation approach, Hussain et al. (2002) found that it was possible to administer testosterone nasally as the testosterone 17β-*N*,*N*-dimethylglycinate hydrochloride prodrug. The solubility of the molecule was increased from 0.01 to 100 mg/mL and the log *P* of the prodrug was increased to 2.4. After nasal administration to rats the pro-drug was rapidly absorbed with an AUC similar to that obtained for testosterone itself. In this example, use of the prodrug made it possible to produce an aqueous formulation for human use with a therapeutically relevant dose (3 mg). The same research group produced water-soluble prodrugs of 17 β-estradiol (in the form of dimethyl butyric and dimethylpropionic esters), and administered them nasally to rats (Al-Ghananeem et al., 2002). The aqueous solubilities of the prodrugs were several orders of magnitude higher than for the parent drug, and both prodrugs were highly bioavailable (~80%). The study also showed that these prodrugs were able to reach the CSF in the brain.

3.1.5.2. Co-solvents. A simple approach to obtaining higher solubility of a drug is to use a mixed solvent system or a co-solvent. Such co-solvents need to be non-toxic, pharmaceutically acceptable, and for nasal delivery, a non-irritant to the nasal mucosa. Solvents often used in nasal drug delivery are glycerol, ethanol, propylene glycol, and polyethylene glycol (PEG). In addition to facilitating drug solubilization, such co-solvents may provide permeation enhancement across certain types of tissue, e.g., skin tissue (Williams and Barry, 2004). However, it is not established that co-solvents known to provide skin penetration will also afford transepithelial permeation enhancement (Birudaraj et al., 2005).

Although the solubility of buprenorphine hydrochloride in water is 16.3 mg/mL, it was considered necessary to increase the solubility of the drug in order to produce a therapeutically relevant formulation (Lindhardt et al., 2000). In a study utilizing sheep, formulations containing 30% PEG 300 (solubility of buprenorphine 25 mg/mL) were administered nasally and compared with similar formulations containing 5% glucose. The higher solubility did not improve the bioavailability, which was about 80% for both formulations. A similar study was performed for melatonin, which has a low solubility in water (2 mg/mL). A nasal dose of 5 mg has been shown to be effective in humans for improving sleep patterns. The solubility was increased to 30 mg/mL by using a 40% PEG 300 solution and a formulation suitable for nasal administration (Bechgaard et al., 1999). Low intranasal irritation was observed for PEG 300 solution in humans.

Jang et al. (2001) evaluated the nasal bioavailability of a 50% ethanolic saline solution of procyclidine (an anticholinergic compound used for the treatment of Parkinson's disease) in rats and dogs. The bioavailabilities were 81.1 and 98.6%, respectively. The solubility of procyclidine in water is 0.025 mg/mL; hence a solubility enhancing approach was needed before nasal

delivery at therapeutically relevant doses could be possible. However, the formulation utilized in this study would most likely cause irritation in the nasal cavity due to the high concentration of alcohol used. No clinical results of such a formulation have been reported.

Epilepsy is presently treated with drugs such as diazepam and clonazepam, which are administered to suppress epileptic convulsions. Given the setting in which these drugs are used, a formulation with a rapid onset of action would be beneficial to the patient. However, these drugs are poorly soluble, and the development of therapeutic nasal formulations requires the use of co-solvents or other solubility-enhancing approaches in order to be feasible. Li et al. (2000) produced formulations containing a mixed solvent system comprised of 30% ethanol, 60% propylene glycol, 10% water, and either 20 mg/mL diazepam or 4.2 mg/mL clonazepam. When dosed in a rabbit model, the formulations demonstrated a T_{max} of <5 min, and a pharmacodynamic response was seen ~1.5 min following the dosing. The tolerance of such formulations in the nasal cavity was not discussed and needs to be evaluated in order to assess the therapeutic utility of this approach.

3.1.5.3. Cyclodextrins. Cyclodextrins are a group of structurally related cyclic compounds formed during bacterial digestion of cellulose. They consist of (α-1,4)-linked α-D-glucopyranose units, comprised of a lipophilic central cavity and a hydrophilic outer surface, and are shaped like truncated cones. The natural α-, β-, and γ-cyclodextrins consist of six, seven, and eight glucopyranose units, respectively. The natural cyclodextrins, especially the β-cyclodextrins have limited aqueous solubility, which can be problematic with respect to developing therapeutically relevant drug formulations. New derivatives of the natural cyclodextrins have been developed that have improved complexation efficiency.

In combination with other drugs, cyclodextrin can form dynamic molecular inclusion complexes in which the lipophilic part of the molecule is incorporated into the lipophilic central cavity of the cyclodextrin. Most drugs form 1:1 complexes with cyclodextrin. In general, the apparent aqueous solubility of lipophilic drugs is increased by this mechanism. Hence, 2-hydroxypropyl β-cyclodextrin increased the solubility of progesterone 88-fold and that of flurbiprofen by 28-fold (Florence and Attwood, 1998). There are examples of currently marketed products that contain cyclodextrin excipients. Most of these products are for oral or parenteral administration, and the cyclodextrin serves to improve solubility or stability. A nasal product (Aerodiol) containing 17-β-estradiol solubilized in dimethyl-β-cyclodextrin is marketed for the treatment of menopausal symptoms. In this case, the dimethyl-β-cyclodextrin was reported to exhibit both solubility enhancing and permeation enhancing properties; the solubilized 17-β-estradiol gave significantly higher nasal bioavailability in rabbits (94.6%) compared to the non-solubilized suspension formulation (25.2%) (Hermens et al., 1990). The IN formulation had only minor adverse effects, was well tolerated, and was as efficient as transdermal and oral formulations of estradiol (Studd et al., 1999; Lopes et al., 2000).

A similar formulation, in which dimethyl-β-cyclodextrin was used to solubilize progesterone for nasal delivery, has been tested in human volunteers and has been shown to result in blood levels of progesterone comparable to those obtained after intravenous administration (Merkus, 1999). In addition to the solubilizing and stabilizing effect of the cyclodextrins, these materials may also have an absorption enhancing effect on the nasal membrane. This effect appears to be most pronounced for dimethyl-β-cyclodextrin (Merkus et al., 1999; Yang et al., 2004).

Loftsson et al. (2001) investigated the solubilization of three benzodiazepines (i.e., alprazolam, midazolam and triazolam) with sulphobutylether-β-cyclodextrin (Captisol®), dimethyl-β-cyclodextrin, and 2-hydroxypropyl-β-cyclodextrin, as well as the effect of pH on the stability constant of the drug/cyclodextrin complex and formation of protonated ring-open drug forms. It was found that cyclodextrin solubilization was enhanced through reversible ring opening of benzodiazepines and ionization of the ring-open form. This effect was most pronounced for midazolam. Midazolam (17 mg/mL) solubilized with Captisol® (14%) was administered nasally to human volunteers. The drug was absorbed rapidly after nasal administration (T_{max} <15 min) and had a bioavailability of 73%.

Hydroxypropyl-β-cyclodextrin has been used to solubilize prostaglandin (Gu et al., 2005). The complex significantly enhanced the solubility and stability of the drug. Solubility was increased from 0.1 to 1.6 mM with the addition of 10 mM hydroxypropyl-β-cyclodextrin. After nasal administration to rats, the pharmacodynamic effect was about 25% of that obtained for an IV injection and the T_{max} was less than 1 min. Of particular interest is a study by Abe et al. (1995), in which derivatives of α, β, and γ-cyclodextrin were used as excipients for IN buserelin. In this study, the purpose of the cyclodextrin was not for aiding drug dissolution, but rather for solubilization of oleic acid, a lipophilic absorption enhancer. Hydroxypropyl-β-cyclodextrin was found to be superior to the other cyclodextrins in this respect. After nasal administration in rats, the bioavailability of the buserelin in combination with oleic acid and cyclodextrin increased compared to the formulation without the cyclodextrin.

Asai et al. (2002) evaluated the effect of water-soluble cyclodextrins on the integrity of the nasal mucusa in rats. After 5 min exposure on the nasal mucosa, no tissue damage was visible for the 1.5% β-cyclodextrin, the 5% hydroxypropyl-β-cyclodextrin, or the 20% hydroxypropyl-β-cyclodextrin, whereas 20% dimethyl-β-cyclodextrin showed severe damage to the membrane, similar to that after exposure to 1% laureth–9 (a non-ionic surfactant) or 1% sodium deoxyglycolate (a bile salt). Neither 30 min nor 60 min exposure to 10% hydroxypropyl-β-cyclodextrin or dimethyl β-cyclodextrin resulted in obvious damage as observed by light microscopy.

3.1.5.4. Choice of salt form. Alternate salt formation is another means of modifying the properties (e.g., physicochemical characteristics and biological performance) of an ionizable drug, and can be used to increase the solubility of poorly soluble drugs, making them more amenable to use in a variety of delivery systems. Unfortunately, no reliable predictions can be made about the relationship between counterion characteristics and prop-

erties of the resulting salt, including solubility (O'Connor and Corrigan, 2001). Hence, the selection of a salt form that may improve the specific properties of a drug is an empirical process. A good example of this is a study on the evaluation of the characteristics of a range of diclofenac salts (O'Connor and Corrigan, 2001). Diclofenac is an acidic compound ($pK_a \sim 3.80$) with very low aqueous solubility (6×10^{-5} M at 25 °C, ~17.8 μg/mL) used for treatment of pain and inflammation in rheumatic disease and for postoperative pain. The sodium salt has an increased solubility of 1.11 mg/mL (~3.49 mM) at neutral pH and is used in marketed tablet formulations in doses up to 150 mg daily. O'Connor and Corrigan (2001) produced a range of amine salts and found that the aqueous solubilities ranged from 3.95 mM (tris(hydroxymethyl)aminomethane salt) to 446 mM (2-(dimethylamino)ethanol or deanol salt), a 113-fold difference in solubility. A correlation was found between the inverse of the salt melting point and the log of the salt solubility, and a log–log relationship was found between salt solubility and the hydrogen ion concentration in the salt solution. A correlation was also found between the free base melting point and the salt melting point.

Nielsen et al. (2005) performed similar studies to evaluate the enhancement of the aqueous solubility of the tertiary amine compound bupivacaine (a local anesthetic) (solubility ~0.4 mM). Two strategies were pursued: alkylation of the amine group in the molecule and choice of the salt form. The salts investigated were chloride, mesylate, formate, acetate, glycolate, and tosylate. The *N*-alkylation and salt formation produced quaternary ammonium salts possessing pH-independent aqueous solubilities that significantly exceeded (up to a factor of 3200-fold at pH 8) that of the parent tertiary amine. However, no information regarding the ability of these compounds to be absorbed in physiologic settings was provided. Another example is improvement in the solubility of piroxicam, a NSAID with a solubility of 0.03 mg/mL in water at 37 °C (Gwak et al., 2005). Since a fast onset of action is essential for the treatment of pain, a faster dissolution rate of tablet formulations was sought. Piroxicam can be ionized to zwitterions that have two pK_a values, 1.86 and 5.46. Three different ethanolamine salts were produced, and dissolution properties and bioavailability after oral administration were evaluated in rats. The mono and di-salts were shown to have the fastest dissolution at pH 6.8, and the mono salt doubled the bioavailability (29.2% compared with the 13.6% bioavailability of the parent drug). Such concepts may have utility in enhancing the absorption of drug compounds formulated for IN delivery.

As far as we are aware, only a single paper in the literature describes the use of different salt forms to enhance drug solubility to improve the nasal formulation of a poorly soluble drug, the acetylcholinesterase inhibitor galantamine (Kays Leonard et al., 2005). The typical oral dose of galantamine hydrobromide (HBr) is 8 mg. Assuming nasal and oral bioavailability are equivalent, the nasal formulation would require a solubility of at least 80 mg/mL in order to apply 8 mg in a spray volume of 100 μL. The solubility of the HBr salt of galantamine is about 35 mg/mL, far below the minimum target concentration. To enhance solubility, the bromide ion was exchanged with either lactate or gluconate, resulting in a solubility of 400 mg/mL for both salt forms. In vivo studies in rats showed bioavailability for nasal administration similar or superior to that for oral administration; therefore salt exchange improves solubility, making IN dosing feasible for this compound and, potentially, for other therapeutically relevant compounds.

In addition, the patent literature contains several examples in which different salt forms are used to enhance solubility. A patent by Illum et al. (2003) describes novel compositions of opioids (especially morphine) for nasal delivery. In certain clinical situations, such as break-through pain, some patients are in need of high concentrations of morphine. The normal salts of morphine, such as sulphate and hydrochloride, are poorly soluble in aqueous solutions (64.5 mg/mL and 57.0 mg/mL, respectively), making it impossible to produce nasal formulations with a sufficiently high concentration to meet this need. Illum et al. (2003) describes the in situ formulation of the methane sulphonate salt of morphine, which has a solubility of about 300 mg/mL morphine base equivalent (equivalent to 400 mg/mL of morphine sulphate), a 6-fold improvement in solubility. A patent by Behl (2003) describes the use of a morphine gluconate salt with a solubility of 250 mg/mL for nasal application. This also represents a considerable improvement in drug solubility. Cancer patients treated with nasally administered morphine gluconate (40 mg) or with morphine methane sulphonate (5–80 mg) with additional absorption enhancers experienced rapid onset of pain relief and good pain scores (Fitzgibbon et al., 2003; Pavis et al., 2002).

However, change in drug salt form to improve solubility in the IN formulation may result in irritation of the nasal tissue, due to the nature of the salt or the degree of hypertonicity. Thus, the selection of the appropriate counterion must be based on local tolerability (e.g., based on in vitro and/or in vivo screening studies), as well as on improving drug solubility.

3.2. Role of transporters, efflux systems

To address the potential role of transporters and efflux systems for intranasal delivery, the literature regarding oral delivery is of interest. Oral absorption in the gastrointestinal tract may be limited due not only to the physicochemical characteristics of the molecule, but also to the coordinated action of intestinal enzymes and efflux transporters (Benet et al., 1996; Wacher et al., 1996). In particular, cytochrome P450 (CYP) 3A4 is located in the epithelial lining of the intestine and has been recognized as contributing significantly to the first-pass metabolism of drugs (Paine et al., 1996). The role of the cytochrome P450 enzyme in terms of nasal absorption of drugs has already been discussed above.

Drug absorption can also be diminished due to efflux transporters such as P-glycoproteins (P-gps). P-gps are large glycosylated membrane proteins found primarily in the apical membrane of epithelial cells of the small intestine and in various other tissues throughout the body (Thiebaut et al., 1987; Schinkel, 1997). Two P-gp genes (coding for MDR1 and MDR3 proteins) are present in humans, whereas rodents have three (coding for MDR1a, MDR1b, and MDR2). These efflux transporter systems actively pump drugs and other compounds from

cells back into the intestinal lumen. It has been shown that a dynamic interplay exists between P-gps and CYP3A in the intestine and the liver, demonstrating that P-gp efflux transport can enhance or impede metabolism by CYP3A (Benet et al., 2004). The P-gps have the ability to transport a diverse range of compounds with no obvious shared structural characteristics. Compounds can range in size from 250 Da (e.g., cimetidine) to about 1900 Da (e.g., gramicidin). Most drugs that are effectively transported are basic or uncharged. All P-gp substrates discovered so far are amphipathic in nature (Schinkel, 1997).

MDR1 (and other transporter systems such as the multidrug resistance associated protein, MRP1) has been found in the human nasal respiratory mucosa, where it can transport a large variety of hydrophobic and amphiphilic substrates, and is considered to be effective in cell detoxification of xenobiotics (Wioland et al., 2000). Another role of MDR1 is the transport of peptides, such as peptide growth factors (Schinkel, 1997). Similarly, these two transporter proteins have been found in olfactory receptor neurons in tadpoles (Manzini and Schild, 2002). However, according to Bremer et al. (1992), the levels of MDR1 in the respiratory epithelium of man are lower (by 250-fold) than the levels in, for example, the liver. Henriksson et al. (1997) have shown that administration of topical steroids in the nasal cavity may increase the expression of P-glycoprotein in the respiratory epithelium.

There are relatively few reports regarding the importance of transporter systems (such as P-glycoprotein) for drug transport across the nasal epithelium. Recently however, several papers have focused on the role of P-glycoproteins in the olfactory epithelium. Graff and Pollack (2003), for example, found that P-gp attenuated the transport of a range of drugs that are substrates for P-gp to brain tissue following nasal administration. Uptake into the brain was enhanced when drugs were administered in combination with the P-gp efflux inhibitor, rifampin. The precise location of the affected efflux system was not identified in these experiments, but it was speculated that the P-gp inhibitor might have had an effect on P-gp in the blood–brain barrier.

Kandimalla and Donovan (2005a,b) conducted in vitro studies using excised bovine olfactory mucosa to explore the transport of the P-gp substrates chlorcyclizine and chlorpheniramine. They found that transport was greater from the serosal to the mucosal side of the tissue than from the mucosal to the serosal side. Moreover, the flux was found to be concentration dependent and saturable. P-gp was visualized in the apical regions of the ciliated epithelial cells and in the submucosal vessels. P-gp and MRP1 inhibitors such as verapamil and quinidine inhibited the P-gp efflux as was the case for metabolic inhibitors such as ouabain and 2,4-dinitrophenol. The authors concluded that the P-gp localized in the olfactory epithelium played a significant role in preventing the drugs from crossing the membrane. Chou and Donovan (1997) believed that the reason chlorcyclizine and chlorpheniramine did not reach the CSF in rats was most likely due to the presence of efflux transporters in the olfactory epithelium. The authors also suggested that the same efflux transporters in the subepithelial lymphatics and vascular endothelium could act as an additional barrier to transport. More recently, the authors reported the presence of P-gp efflux transporters in both the respiratory epithelium and in the olfactory epithelium as evidenced by histological and immunohistochemical analysis of excised bovine tissues. The concentration of P-gp was much higher in the olfactory region than in the respiratory region. They demonstrated that efflux of the model drug substrate etoposide by P-gp was significant, and that this efflux could be decreased by inhibition with 2,4-DNP and quinidine.

In an interesting paper, Graff and Pollack (2005a) investigated in detail the influence of the efflux P-gp inhibitor, rifampin, on the transport of the drug verapamil from the nasal cavity and from the systemic circulation into the brain of mice. Rifampin was administered both nasally and intravenously. Regardless of delivery route, the compound inhibited the efflux of verapamil whether the drug was given nasally or by brain perfusion. The best inhibitory effect was found when both the drug and the inhibitor were given nasally, resulting in nearly 100% inhibition. The inhibition effect of rifampin was found to be most efficient after nasal administration, for which a much lower concentration (400-fold lower) was needed than after intravenous administration. Because the total amount of drug given via brain perfusion or via nasal delivery is not reported in the paper, the relative ratios of drug to inhibitor cannot be determined. Although the study showed that nasal administration of rifampin increased the brain uptake of a systemically administered drug, the authors did not indicate the degree of systemic distribution of the drug or inhibitor after nasal administration. Therefore, it is difficult to deduce the extent to which inhibition resulted from a systemic effect rather than a nasal (to-brain) effect. For nasally administered rifampin, the concentration in the brain was located more rostrally than after systemic administration, in which the brain distribution was more uniform. Whether this had an effect on the inhibition efficiency was difficult to deduce from the study.

Immunohistochemical studies localized P-gp to endothelial cells lining the olfactory bulb and the olfactory epithelium (Graff and Pollack, 2005a). It was concluded that P-gp localized to both of these sites plays a role in the attenuation of drug uptake into the brain. Though it was suggested that the inhibitor might be able to reach the BBB after nasal administration, no indication of the pathway was given.

An additional publication by Graff et al. (2005) used pharmacokinetic modeling to investigate the pharmacokinetics of uptake and distribution in the brain of model drugs that are substrates for P-gp. In this model, P-gp-mediated efflux was larger from the nasal epithelium than from the olfactory epithelium, mainly due to the larger surface area at the former site. The study confirmed the earlier findings that P-gp is functional at the olfactory epithelium and serves to attenuate brain uptake of drugs after nasal administration. It was also shown that P-gp operates throughout the brain to efflux substrates during rostral to caudal distribution.

Yet another study by Graff and Pollack (2005b) further investigated the transport of an efflux P-gp inhibitor, rifampin, from the nasal cavity into various brain tissues and its effect on the increase in brain uptake of loperamide, a known substrate for P-gp, administered by the intravenous route. Loperamide is

unable to cross the BBB due to P-gp mediated efflux. The experiment showed that nasally administered rifampin modulated P-gp efflux of systemically administered loperamide beyond the olfactory bulb, but to a lesser degree than rifampin administered intravenously. However, the nasal dose of the inhibitor was significantly lower than what was dosed intravenously. The authors suggest that the nasally administered inhibitor is able to reach the BBB and inhibit P-gp at this site.

The existence of transporter systems in the nasal cavity and their effect on absorption of drugs from the nasal mucosa into the systemic circulation and on transport into the CNS is a very new area of research. Further developments to understand the role of these systems in achieving therapeutically relevant concentrations of drug in the CNS may have an important impact on the development of drugs formulated for IN delivery.

4. Concluding remarks

In order to ascertain whether IN delivery is appropriate for any given drug, various facets of the drug's characteristics and intended use need to be considered. IN delivery may be suitable for either topical or systemic delivery. In the latter case, rapid onset is a key feature of the typical IN PK profile and may provide a distinct advantage in certain circumstances, e.g., pain management. Another application for IN dosing is for vaccine therapeutics. IN delivery can be utilized for high-molecular-weight drugs such as peptides and proteins, however, systemic bioavailability is dramatically dependent upon the presence of permeation enhancers. IN administration may be particularly attractive for therapies requiring chronic dosing. For many drugs, IN administration provides a good alternative to the invasiveness of injections and to oral delivery, which may be associated with problems such as poor bioavailability and the potential for GI-related side-effects. These characteristics should be considered by the formulation scientist when considering IN dosing for new chemical entities and for product life extension.

Development of an IN dosage form can be undertaken once the desirability and feasibility of this approach has been established based on considerations discussed herein. A thorough guidance on development of IN products is beyond the scope of this review. As a starting point, the interested reader is encouraged to consult relevant regulatory guidances. For instance, general regulatory guidances are available for developing IN products in Europe (EMEA Committee for Medicinal Products for Human Use, 2006) and the U.S (FDA Center for Drug Evaluation and Research, 2002). Other examples include guidances for developing generic products for local IN delivery (FDA Center for Drug Evaluation and Research, 1999) and new nasal sinusitis products (FDA Center for Drug Evaluation and Research, 2006) in the U.S.

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