

Nasal drug delivery—possibilities, problems and solutions

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Abstract

This paper discusses the problems associated with nasal drug delivery and how it is possible, sometimes by means of quite simple concepts, to improve transport across the nasal membrane. In this way it is feasible to deliver efficiently challenging drugs such as small polar molecules, peptides and proteins and even the large proteins and polysaccharides used in vaccines or DNA plasmids exploited for DNA vaccines. The transport of drugs from the nasal cavity directly to the brain is also described and examples of studies in man, where this has been shown to be feasible, are discussed. Recent results from Phase I/II studies in man with a novel nasal chitosan vaccine delivery system are also described. Finally, the author's thoughts about the future for nasal drug delivery are also depicted.

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1. Introduction

Conventionally, the nasal route of delivery has been used for delivery of drugs for treatment of local diseases such as nasal allergy, nasal congestion and nasal infections. Recent years have shown that the nasal route can be exploited for the systemic delivery of drugs such as small molecular weight polar drugs, peptides and proteins that are not easily administered via other routes than by injection, or where a rapid onset of action is required [1]. Marketed products include a range of anti-migraine drugs such as sumatriptan from GlaxoSmithKline, zolmitriptan from AstraZeneca, ergotamine from Novartis and butorphanol from BristolMyersSquibb, as well as a range of peptides, such as calcitonin marketed by Novartis, desmopressin from Ferring and buserelin

from Aventis (Table 1). A wide range of nasal products is in development, mostly aimed for exploiting the advantage of a rapid onset of action when administered via this route, for example for the treatment of pain, nasal morphine and ketamine and for the treatment of erectile dysfunction, nasal apomorphine. Lately the use of the nasal route for delivery of vaccines, especially against respiratory infections such as influenza, has also attracted interest from the pharmaceutical companies specialising in vaccine delivery. This is due to the possibility of obtaining not only a systemic but also a local immune response. Hence, the first nasal influenza product from the Swiss company, Berna Biotech reached the European market in 2001 (but is now withdrawn due to potential toxicological problems) and a second product from Aviron is due to be launched in 2003 [2]. The present paper discusses the problems associated with nasal delivery of drugs and how barriers to the nasal absorption of especially

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Table 1

Nasal delivery of peptides and proteins

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- Nasal salmon calcitonin
 - Marketed by Novartis
 - Novel nasal formulations under development by other pharmaceutical companies
 - Nasal desmopressin
 - Marketed by Ferring and partners
 - Nasal buserelin
 - Marketed by Aventis
 - Nasal nafarelin
 - Marketed by Searle
 - Nasal PTH, nasal leuprolide, nasal insulin, nasal interferon, etc.
 - In clinical trials
-

challenging drugs can be overcome. It also highlights the possibilities for achieving novel targets (Brain, CSF) using this route of delivery.

2. The nasal cavity

The nasal cavity has an important protective function in that it filters, warms and humidifies the inhaled air before it reaches the lower airways. Any inhaled particles or microorganisms are trapped by the hairs in the nasal vestibule or by the mucus layer covering the respiratory area of the nasal cavity. Due to the mucociliary clearance mechanism, the mucus layer will gradually carry such particulates to the back of the throat, down the oesophagus and further into the gastrointestinal tract. Furthermore, the nasal mucosa has a metabolic capacity that will help convert endogenous materials into compounds that are more easily eliminated.

A midline septum divides the human nasal cavity into two non-connected parts. Each part consists of three regions: Firstly, the vestibule consisting of the region just inside the nostrils with an area of about 0.6 cm². Secondly, the olfactory region, which in man is situated in the roof of the nasal cavity and only covers about 10% of the total nasal area of 150 cm² as opposed to for example in the dog where the area constitute 77% of the total nasal cavity, and thirdly the respiratory region which constitute the remaining region. The respiratory region contains the three nasal turbinates, the superior, the middle and the inferior which project from the lateral wall of each half of the nasal cavity. The presence of these turbinates creates a turbulent airflow through the

nasal passages which ensures a better contact between the inhaled air and the mucosal surface. Similar, more or less complex turbinate structures are present in all animal models normally used for nasal delivery studies.

The nasal vestibule is covered with stratified squamous epithelium which gradually changes posteriorly into a pseudostratified columnar epithelium that covers the respiratory epithelium. The respiratory epithelial cells are covered by microvilli and the major part of these cells is also covered with cilia. These cilia, which are long (4–6 µm) thin projections, are mobile and beat with a frequency of 1000 strokes per min. The beat of each cilium consists of a rapid forward movement, where the cilium is stretched and the tip of the cilium reaches into the mucus layer and carries this forward followed by a slow return beat, where the cilium is bent and moves in the sol layer that lies beneath the mucus layer. In this way the mucus layer is propelled in a direction from the anterior towards the posterior part of the nasal cavity. The mucus flow rate is in the order of 5 mm per min and hence the mucus layer is renewed every 15–20 min.

3. Barriers to nasal absorption

Lipophilic drugs are generally well absorbed from the nasal cavity with the pharmacokinetic profiles often identical to those obtained after an intravenous injection and bioavailabilities approaching 100%. A good examples of this is the nasal administration of fentanyl where the T_{max} for both intravenous and nasal administration have been shown to be very

rapid (7 min or less) and the bioavailability for nasal administration was near 80% [3]. However, despite the large surface area of the nasal cavity and the extensive blood supply, the permeability of the nasal mucosa is normally low for polar molecules, to include low molecular weight drugs and especially large molecular weight peptides and proteins. For small polar drugs the bioavailabilities are generally in the region of 10% and for peptides such as calcitonin and insulin normally not above 1%. It is interesting to note that the nasal sumatriptan product on the market has a pharmacokinetic profile not dissimilar to that obtained after oral administration and a bioavailability of 15.8% as compared to 14.3% after oral administration [4]. It is evident from the published results that only a minor part of the absorption is due to absorption from the nasal cavity (small peak at 30 min) and that a $C_{\max}$ obtained after 2 h is due to oral absorption.

The most important factor limiting the nasal absorption of polar drugs and especially large molecular weight polar drugs such as peptides and proteins is the low membrane permeability. Drugs can cross the epithelial cell membrane either by the transcellular route, exploiting simple concentration gradients, receptor mediated transport or vesicular transport mechanisms or by the paracellular route through the tight junctions between the cells. Polar drugs with molecular weights below 1000 Da will generally pass the membrane using the latter route, since, although tight junctions are dynamic structures and can open and close to a certain degree, when needed, the mean size of the channels is in the order of less than 10 Å and the transport of larger molecules is considerably more limited [5,6]. Larger peptides and proteins have been shown to be able to pass the nasal membrane using an endocytotic transport process but only in low amounts [7,8].

Another factor of importance for low membrane transport is the general rapid clearance of the administered formulation from the nasal cavity due to the mucociliary clearance mechanism. This is especially the case for drugs that are not easily absorbed across the nasal membrane. It has been shown that for both liquid and powder formulations, that are not mucoadhesive, the half life of clearance is in the order of 15–20 min [9,10]. It has further been suggested that the deposition of a formulation in the

anterior part of the nasal cavity can decrease clearance and promote absorption as compared to deposition further back in the nasal cavity [11]. Most nasal sprays of various makes have been shown to deliver the formulation to a limited area in the anterior part of the nasal cavity as opposed to nasal drops which will be delivered to a larger area further back in the nasal cavity.

The nasal absorption of such polar drugs can be greatly improved if administered in combination with absorption promoting agents. Agents described in the literature for nasal drug delivery have included surfactants such as laurth-9, bile salts and bile salt derivatives such as sodium taurodihydrofusidate, fatty acids or fatty acid derivatives, phospholipids as well as various cyclodextrins [1]. These enhancer systems work by a variety of mechanisms but generally change the permeability of the epithelial cell layer by modifying the phospholipid bilayer, leaching out protein from the membrane or even stripping off the outer layer of the mucosa. Some of these enhancers also have an effect on the tight junctions and/or work as enzymatic inhibitors. For such enhancing agents greatly enhanced bioavailabilities have been obtained, even for larger peptides such as insulin at least in animal models. Less success has been achieved in Phase 1 clinical trials, very often due to a lesser effect in humans or to the irritancy of the enhancer material in the nasal cavity. In animal studies it has been shown for a range of enhancing agent that there is a direct correlation between absorption enhancing effect (bioavailability) obtained and the damage caused to the nasal membrane [1]. This is particularly true for surfactant materials and bile salts. For other enhancers, such as some of the cyclodextrins, chitosan and selected phospholipids the absorption enhancing effect outweighs any damage caused to the mucosa. Hence, it is of great importance that one considers carefully the choice of an absorption enhancer for a nasally delivered drug that is not readily absorbed especially in terms of potential nasal and systemic toxicity.

Another contributing (but normally considered less important) factor to the low transport of especially peptides and proteins across the nasal membrane is the possibility of an enzymatic degradation of the molecule either within the lumen of the nasal

cavity or during passage across the epithelial barrier. These sites both contain exopeptidases such as mono- and diaminopeptidases that can cleave peptides at their N and C termini and endopeptidases such as serine and cysteine, that can attack internal peptide bonds [12]. A range of studies has been carried out *in vitro* using tissue homogenates or nasal wash or *in vivo* in animal models to assess the possible effect of such enzymes on the stability of peptides [13–15]. However, it is often difficult to truly evaluate the relevance of the experimental set up to the real *in vivo* situation in man. Very often the exposure of the peptide to the enzyme has been overestimated and for some peptides degradation half-lives of less than 30 min have been quoted.

4. Chitosan

In designing effective delivery systems for a range of challenging molecules especially biopharmaceuticals, we have tried to take into account the factors that can effect nasal absorption. Absorption has been improved by using a number of strategies taking into account the drug, the disease and the destination. We have concentrated on overcoming two of the main barriers to effective absorption, namely transport across the epithelial membrane and the rapid mucociliary clearance of the formulation. We have focused upon a strategy employing the use of bioadhesive nasal delivery systems that provide the prolonged contact between the drug formulation and the absorptive sites in the nasal cavity by delaying the mucociliary clearance of the formulation. Such bioadhesive systems can be in the form of powders as well as liquids or liquid gelling systems. The powders can be administered as freeze dried or spray dried particles or microspheres. For such systems we have found starch and chitosan to be preferred materials in terms of their efficiency. Most recently we have focused our work on the use of the bioadhesive polysaccharide chitosan and the development of a range of delivery systems based on this material [1].

Chitosan is a positively charged linear polysaccharide that is bioadhesive and able to interact strongly with the nasal epithelial cells and the overlying mucus layer thereby providing a longer contact time for drug transport across the nasal

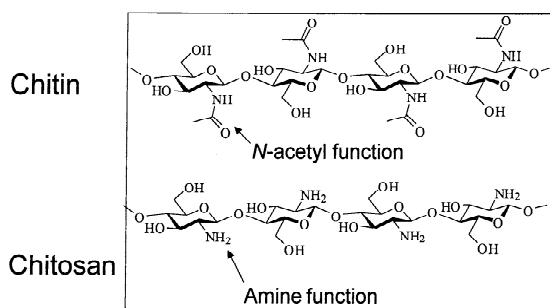


Fig. 1. Chitin and chitosan.

membrane, before the formulation is cleared by the mucociliary clearance mechanism [1,10,16]. In addition, chitosan has been shown (in Caco-2 cell culture studies) to increase the paracellular transport of polar drugs by transiently opening the tight junctions between the epithelial cells [17,18]. Chitosan is produced from chitin present in shells of crustaceans by a process of deacetylation and is available in a range of molecular weights and degrees of deacetylation. The structure of chitosan is shown in Fig. 1. The type of chitosan most often employed for nasal delivery is a chitosan glutamate salt of a mean molecular weight around 250 kDa and a degree of deacetylation of more than 80%. This chitosan salt is soluble in water up to a pH of about 6.5.

5. Nasal delivery of polar drugs

Various pre-clinical and clinical studies have been carried out in animal models and in human volunteers and patients for the development of therapeutically relevant nasal formulations of small polar drugs and a range of peptides and proteins using the chitosan nasal delivery system. Morphine is a good example, since for the management of breakthrough pain it is important to obtain a pharmacokinetic profile where an early peak plasma level is achieved and thereby a rapid onset of action is provided. This is not achieved at present. Breakthrough pain is generally treated with oral morphine solution or similar drugs where the plasma peak level is not achieved before 30–45 min after administration. The morphine opioid is relatively polar ($\log P=0.89$) and when administered nasally in a simple formulation to

human volunteers the pharmacokinetic profile resembles that following an oral administration with a $T_{\max}$ about 40 min [19]. Only low amounts are absorbed nasally of the order of 5–10%.

However, when morphine is administered in a simple chitosan solution formulation a five to six-fold increase in bioavailability to about 60% is achieved with a $T_{\max}$ of 15 min or less [20]. The shape of the plasma curve has been found to be similar to that obtained for the intravenous administration of morphine (Fig. 2). Furthermore, the metabolic profiles obtained after intravenous and nasal administration of the respective formulations were very similar with comparable ratios between the plasma levels of morphine-3-glucuronide and morphine-6-glucuronide and similar low levels of both metabolites as compared to the five times higher levels of both after oral administration. It was also found that the nasal formulation was well tolerated with minimal side effects of less than 30% of total maximal scores. The effect of the nasal chitosan–morphine formulation on the management of breakthrough pain has been investigated in a pilot study in 14 cancer patients. This demonstrated that within 5

min the patients experienced a significant decrease in pain intensity and an associated increase in pain relief [21]. The chitosan–morphine nasal product is now in Phase II clinical trials both in the USA and in the UK and is expected to reach the market within the next 2 years.

Apart from the small molecular weight drugs, the chitosan delivery system, whether in the form of a solution or a powder, has also been shown to be very effective in delivering peptides such as leuprolide (1300 Da), salmon calcitonin (S-CT) (3500 Da) and parathyroid hormone (PTH) (4000 Da) across the nasal membrane. Nasal bioavailabilities of around 20% or more have been obtained in clinical trials for these drugs. Generally, it has been shown that chitosan powder formulations, whether in the form of microspheres or powders, can in many instances be able to provide a better absorption promoting effect than chitosan solutions. Hence, for the LHRH analogue, goserelin it was found in the sheep model that a chitosan microsphere formulation was able to provide bioavailabilities in the order of 40% relative to an intravenous injection [22]. For some drugs, such as PTH, a chitosan powder formulation is also

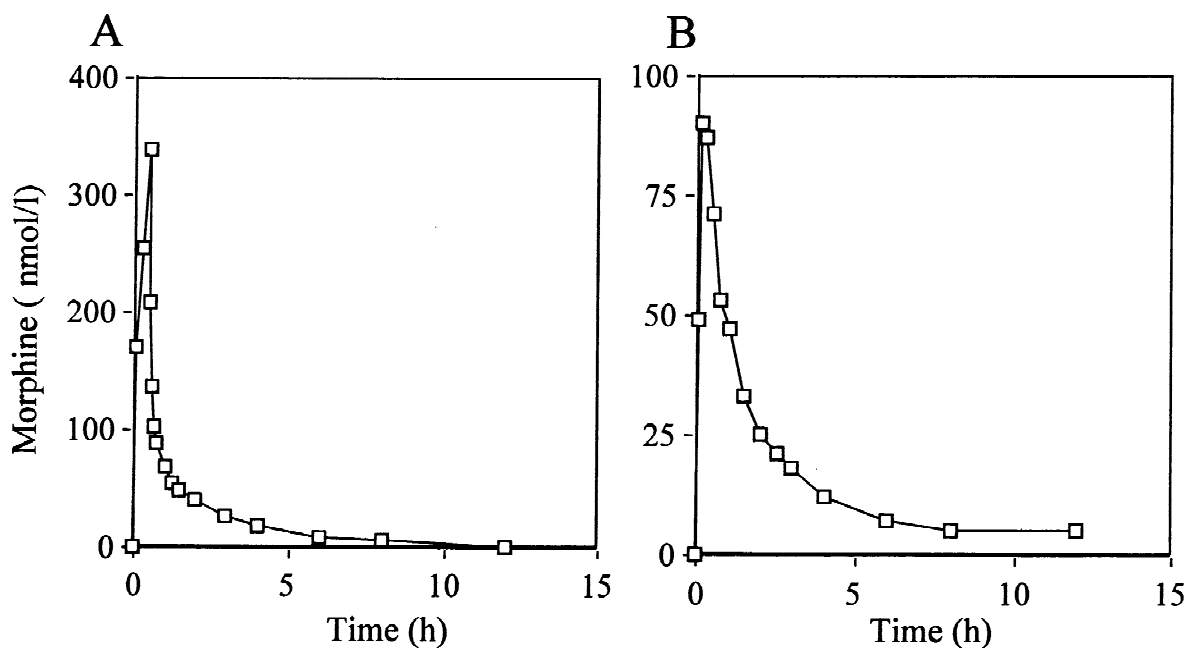


Fig. 2. A comparison between the pharmacokinetic profile of morphine (10 mg) administered as a slow (30 min) infusion (A) and morphine administered nasally in a chitosan solution formulation (B).

the most appropriate formulation due to stability problems encountered with PTH solution formulations.

6. Nose to brain delivery

Recent developments have highlighted the possibility of exploiting the nasal route for direct transport of drugs from nose to brain in man. Hence, Frey [23] declared that by nasally administering insulin-like growth factor (IGF-1) the drug could bypass the blood–brain barrier and reach the central nervous system (CNS) directly from the nasal cavity. Reports in the literature of studies in animal models and in man have shown this to be a distinct possibility with results showing the uptake of drugs into the cerebrospinal fluid and the brain tissue being dependent upon molecular weight and the lipophilicity [24–28]. In mice, it was shown recently that [^3H]-dopamine reached the right olfactory bulb after nasal administration into the right nostril and that at 4 h the concentration in the bulb was 27 times higher than that in the left olfactory bulb [29]. Following intravenous administration the uptake into the brain was low. The paper did not disclose the total

quantity that actually reached the brain. However, according to previous studies in animal models it has been shown that the total amount appearing in the brain tissue seldom amount to more than nM quantities or a bioavailability of 0.01–0.1% [24].

The routes by which nasally delivered drugs can reach the cerebro-spinal fluid (CSF), which surrounds the brain and the actual brain tissue, is depicted in Fig. 3. The thickness of the individual arrows indicates the likelihood of drugs actually exploiting this route of transport. When administered nasally the drug formulation will normally be cleared rapidly from the nasal cavity into the gastrointestinal tract by the mucociliary clearance system. A quantity of the drug will (dependent on the lipophilicity and molecular weight) be absorbed across the nasal mucosa and reach the systemic circulation from where it will be eliminated via normal clearance mechanisms. The drug can also follow the systemic pathway to reach the brain by crossing the blood brain barrier but the use of this pathway is highly dependent on the properties of the drug. Of special interest is the transport of drug across the olfactory region in the nasal cavity directly into the brain tissue (e.g. olfactory bulb) or the CSF. Due to the normal replacement of the CSF (four to five times

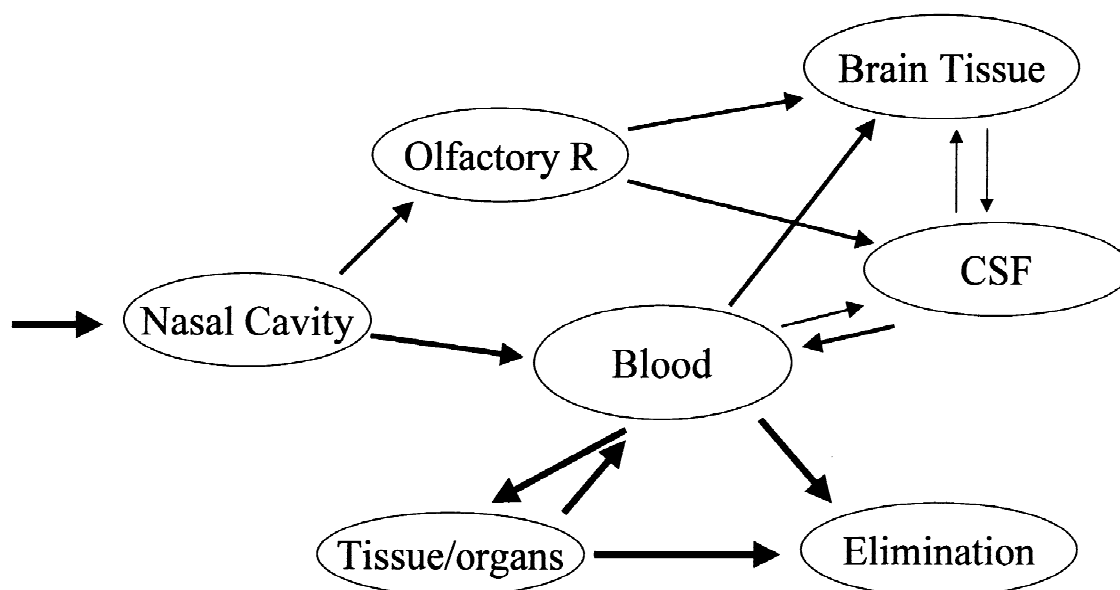


Fig. 3. Possible routes of transport between the nasal cavity and the brain and CSF.

daily), the drug will also gradually be eliminated from the CSF into the blood.

The olfactory epithelium is situated between the nasal septum and the lateral wall of each side of the two nasal cavities and just below the cribriform plate of the ethmoid bone separating the nasal cavity from the cranial cavity. The olfactory epithelium is a modified form of respiratory epithelium in that it is a pseudostratified epithelium that, apart from the supporting epithelial cells with villi and basal cells, also contains olfactory receptor cells. The olfactory receptor cells are bipolar neurones with a single dendritic process extending from the cell body to the free apical surface where it ends in a small knob carrying non-motile cilia. At the basal end the neuron ends in a fine non-myelated axon that joins with other axons into a bundle surrounded by glial cells (and CSF) and penetrates into the cranial cavity through small holes in the cribriform plate.

In order for a drug to reach the CNS from the nasal cavity, it will have to cross the olfactory membrane and depending on the pathway used also the arachnoid membrane surrounding the arachnoid space containing the CSF. The drug can cross the olfactory pathway by one or a combination of pathways. Firstly, the drug can use a transcellular pathway, where the drug is transferred by receptor mediated endocytosis, fluid phase endocytosis or by passive diffusion. This pathway is especially suited for small lipophilic molecules or large molecules. Secondly, the drug can use the paracellular pathway by passing through the tight junctions or through open clefts in the membrane. This pathway is especially suited for smaller hydrophilic molecules. Thirdly, the drug can be transported through the olfactory neuron cells by intracellular axonal transport primarily to the olfactory bulb.

In our own studies we have concentrated on the mechanisms and transport pathways in order to elucidate ways of improving the rate and quantity of drug reaching the brain. Hence, we have investigated the transport of a reasonable hydrophilic zwitterionic drug across the nasal membrane into the CNS using the rat model. The results have shown that the uptake of the drug into the olfactory lobe was significantly higher after nasal administration than after intravenous administration. The AUC for the olfactory lobe was six times larger after nasal administration as

compared to intravenous administration for the first 30 min, whereas the AUC for the plasma was 2.5 times lower. This showed that the drug was transported across the nasal membrane into the olfactory lobe. Whole body autoradiography of the rat at 1, 5, 10 and 30 min after administration showed a distinct increase in radioactivity of the brain area indicating either an increasing brain tissue uptake or an increasing CSF uptake. The CSF concentration was not measured. Microautoradiography of the olfactory region of the rat showed the presence of drug molecules along the olfactory neuron bundles. This can either indicate transneural transport or transport via the CSF surrounding the bundles. However, considering that transneural transport of drugs is a slow process it is most likely that the drug had crossed the olfactory region by means of one of the other transport mechanisms and reached the CSF and the olfactory bulb.

Evidence that nose to brain transport is also taking place in man is steadily accumulating in various reports and papers [25–27,30–34]. Table 2 gives an overview of some of the latest studies performed in man. The first group of studies mostly comprised an investigation of the pharmacodynamic effect on the CNS in terms of specific effects on the event-related potentials during the subject's performance of an auditory (or similar) attention task (oddball task) comparing drug administration via nasal and intravenous routes of delivery. As an example, Pietrowsky [30] administered cholecystokinin-8 (CCK) to 20 healthy volunteers in a placebo controlled, double blind, cross-over study by the nasal and the intravenous route and recorded the auditory event-related potentials (AERP) during the volunteer's performance of an oddball task. Plasma CCK concentrations were similar after both routes of administration. Nasal administration of CCK markedly increased the P3 complex of the AERP, whereas this effect was not seen after intravenous administration. It was concluded that this effect was due to a direct, most likely extracellular pathway, between the olfactory region in the nasal cavity and the brain.

The first attempt at radioisotopic assessment of the integrity of the nose to brain barrier in man was published by Okuyama [31]. A mixture of ^{99m}Tc -DTPA and hyaluronidase was sprayed onto the olfactory mucosa of anosmic patients and the cere-

Table 2

Studies indicative of nose to brain drug transport in man

-
- *Functional evidence of facilitated transport to the brain was provided by changes in event related potential during performance of an oddball task*
 - Arginin–vasopressin ($n=15$) [43]
 - Cholecystokinin-8 ($n=20$) [30]
 - Angiotensin II ($n=12$) [32]
 - Insulin ($n=18$) [44]
 - Adrenocorticotropin 4-10 ($n=54$) [45]
 - Insulin ($n=12$) [34]
 - *Direct evidence of nose to brain uptake*
 - ^{99m}Tc -DTPA–hyaluronidase [31]
 - *Direct evidence of nose to CSF uptake*
 - Insulin ($n=8$) [26]
 - Apomorphine ($n=5$) ? [25]
 - Melatonin/hydroxycobalamin ($n=2$) [46]
-

bral radioactivity measured. A significant rise in cerebral radioactivity was observed 5 min after application of the radioisotope. More recent studies have not only studied the effect on brain potentials but have also compared specific drug concentrations in the CSF after nasal and intravenous administration. Hence, in a pilot study in eight healthy volunteers Fehm et al. [26] reported a distinct accumulation of insulin in the CSF sampled from a spinal tap after a single nasal administration of 40 IU insulin. No rise was seen in serum levels. It is well known that without the addition of an absorption enhancer insulin does not easily cross the nasal membrane to enter the blood stream so it is unlikely that the insulin reached the brain via the blood–brain barrier.

Recently, it was announced in a press release that a Phase 1 clinical study in human volunteers had shown that at 20 min after the nasal administration of apomorphine the concentration of the drug in the CSF was equivalent to 27–44% of the concentration found at the same time in the plasma, whereas after subcutaneous administration the CSF levels were equivalent to only 2.5–4.3% of the plasma levels [25]. The CSF was sampled by spinal tap. Although this study does indicate that a direct transport from nose to the CSF may have taken place the study lacks information on CSF levels after an intravenous injection of apomorphine to give an indication of apomorphine's ability to cross the blood–brain barrier. Furthermore, the subcutaneous results were

taken from the literature and injections were not performed in the same group of people.

It is evident that in situations where it is necessary to target receptors in the brain, as for example for Parkinson's disease, treatment of Alzheimer's disease or the treatment of pain, a specific delivery to the CNS would be beneficial. In such situations efforts should be given to the development of delivery systems capable of increasing the fraction of the drug that reaches the CNS after nasal delivery. However, in other situations, when a transport of nasally administered drugs to the brain may not be beneficial or even harmful to the patient, it is necessary to ensure that the transport of the drug to the blood stream is maximised. Very little effort has so far been directed to the possibility of enhancing or decreasing the transport to the brain via the nose by means of appropriate delivery systems. This is an area that warrants further investigation.

7. Nasal delivery of vaccines

Many diseases, such as measles, pertussis, meningitis and influenza are associated with the entry of pathogenic microorganisms across the respiratory mucosal surfaces and are hence good candidates for nasal vaccines. The main reasons are given in Table 3. It is well established that nasally administered vaccines, especially if based on attenuated live cells or adjuvanted by means of an immunostimulator or a

Table 3

Main reasons for exploiting the nasal route for vaccine delivery

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- The nasal mucosa is the first site of contact with inhaled antigens
 - The nasal passages are rich in lymphoid tissue (Nasal Associated Lymphoid Tissue-NALT)
 - NALT is known as Waldeyer's ring in humans
 - Adenoid or nasopharyngeal tonsils
 - Bilateral lymphoid bands
 - Bilateral tubal and facial or palatine tonsils
 - Bilateral lingual tonsils
 - Creation of both mucosal (sIgA) and systemic (IgG) immune responses
 - Low cost, patient friendly, non-injectable, safe
-

delivery system, can induce both mucosal and systemic (i.e. humoral and cell-mediated) immune-responses. The nasal passages are rich in lymphoid tissues and the target site for nasally administered vaccines in man is considered to be nasal-associated lymphoid tissue (NALT) which is situated mainly in the pharynx as a ring of lymphoid tissue and named Waldeyer's ring.

A range of different nasal vaccine systems has been described in the literature either using live or attenuated whole cells, split cells, proteins or polysaccharides and with and without various adjuvants and delivery systems [35–37]. Some of these systems were shown to be efficient not only in mice but also in man [38]. It is interesting to note that Van Ginkel et al. [39] in a recent paper found that GM1-binding molecules like cholera toxin (CT) and cholera toxin-B subunit (CT-B) that are potent adjuvants, targeted the olfactory nerves/epithelium after nasal administration and were transported in a retrograde manner to the olfactory bulbs. CT and CT-B promoted the uptake of vaccine proteins (such as tetanus toxoid (TT)) into the olfactory neurones whereas TT without the adjuvant did not penetrate into the CNS after nasal administration.

The chitosan nasal delivery system has been tested with three different vaccines, namely for influenza, pertussis and diphtheria in various animal models and in man. It has been shown that chitosan significantly enhances the immune response obtained from influenza vaccine when given nasally to mice, with IgG levels similar to those obtained after parenteral application and significant IgA levels both in the nasal fluid and in the lungs. Lymphocytes secreting antibody specific for HA were recovered from nasal mucosa in all groups of animals. Recently, a Phase 1 clinical trial of nasal chitosan influenza

vaccine has shown promising results, with more than four fold increase in HI values relative to pre-dose HI values and HI values of 40 or higher indicative of protective levels of the antigen. Levels were similar for IM conventional influenza vaccine and nasal administration of the chitosan-influenza vaccine [2]. Similarly, in studies in naïve guinea pigs it was shown that diphtheria vaccine (CRM197) administered nasally in a chitosan formulation provided significantly higher serum IgG titres than the levels obtained for the nasal formulation without chitosan. For the nasal chitosan solution formulation the IgG titres were similar to those obtained after an adjuvanted intraperitoneal injection of CRM197, whereas for the nasal chitosan powder formulation the levels were significantly higher. Similar results were obtained for the nasal wash sIgA levels, except that sIgA levels were lower than for both the nasal chitosan solution and the chitosan powder CRM197 formulations [2]. In a second study guinea pigs were primed with a low dose of subcutaneous diphtheria toxoid and left for 126 days for the titres to decrease to levels resembling background titres in adults vaccinated as children against diphtheria. The animals were vaccinated with nasal chitosan CRM197 solution or powder formulations or with a CRM197 alum formulation given subcutaneously. Anti-diphtheria toxin neutralising antibodies were measured by Vero cell assay and detected in serum in all guinea pigs. The levels after nasal administration with both chitosan formulations were similar to or slightly higher than those obtained after subcutaneous injection. A challenge test in these primed animals showed that the nasally vaccinated animals were as well protected as the animals given the subcutaneous injection [2]. In a three way study in 25 adult human volunteers all previously (>5 years

ago) vaccinated against diphtheria, one group ($n=10$) was vaccinated with intranasal CRM197-mannitol powder, the second group ($n=10$) with CRM197-chitosan powder and the third group ($n=5$) with conventional intramuscular diphtheria vaccine (Dip/vac/ads) at days 1 and 24. The results showed that the nasal chitosan formulation was superior in terms of induced neutralising antibody levels, and sIgA levels as compared to the nasal formulation without chitosan and in terms of sIgA levels as compared to the conventional diphtheria vaccine. The nasal chitosan formulation induced a similar type Th1 and a superior type Th2 response to the conventional diphtheria vaccine. The data clearly indicated that the intranasal CRM197-chitosan vaccine was effective, protective and also induced cellular immunity against diphtheria toxin. It could be concluded from this study that the nasal chitosan powder formulation has an excellent potential for development as a booster diphtheria vaccine [40]. It is suggested that the chitosan enhances the immunity of the vaccine by providing longer residence times in the nasal cavity and by opening transiently the tight junctions between the mucosal cells allowing the vaccine better access to the lymphoid tissue.

Due to its positive charge chitosan has a great potential for complexation with negatively charged DNA plasmids and self-assembling into nanoparticulate systems for improved delivery of DNA. It is possible, by adjusting the ratio between chitosan and DNA, to produce nanoparticles with sizes from 20 to 500 nm and carrying different surface charges (from near neutral to highly positive zeta potentials). We have exploited this concept for the nasal delivery of DNA plasmid expressing epitopes of respiratory syncytial virus (RSV) in order to produce an effective vaccine for this severe illness of new born and young children [41]. In a first study in mice it was shown that nasal immunisation with a nanoparticle complex of chitosan–DNA encoding the CTL epitope from the M2 protein of RSV induced CTL responses comparable to those achieved after intradermal DNA immunisation. In a second study in mice the effect of immunisation with peptides in a chitosan–DNA complex formulation was assessed in a virus challenge experiment. It was found that a significant reduction in the virus load was achieved after intranasal immunisation with the chitosan–

DNA complex formulation compared to the controls. The reduction in virus load was comparable to that induced following subcutaneous immunisation with the positive control (peptide F:M2-9) and intradermal immunisation with the naked DNA. No reduction in the virus load was observed in both of the negative controls. Similarly, a challenge study performed in mice primed with an IM dose of naked DNA expressing haemagglutinin (HA) and nuclear protein (NP) of the influenza A virus and boosted with a nasal or IM chitosan–DNA vaccine showed higher antibody response and significantly reduced virus shedding after challenge as compared to naked DNA [2].

Thus, vaccination by the intranasal route with the chitosan–DNA nanoparticle complexes may well be an attractive and efficient alternative to parenteral administration. Interestingly, Roy et al. [42] have recently evaluated a chitosan based oral DNA vaccine system for vaccination against peanut allergy. A good antigen expression and protection to challenge was achieved in the allergen test system.

8. The future

In the near future we should expect to see a range of novel nasal products reaching the market. It is envisaged that these products will in the first instance primarily comprise products for crisis treatment such as treatment of acute pain, panic attacks, sleep induction, erectile dysfunction, nausea, heart attacks, Parkinson's disease, etc. Further in the future we should also see improved nasal products for treatment of long term illnesses such as diabetes, growth deficiency, osteoporosis, endometriosis, fertility treatment. At present the required drugs are usually either given by injection, have a not totally appropriate plasma profile or display unwanted pharmacological effects due to inappropriate dosing regimens. In the area of vaccines the future should also see a range of novel nasal vaccines introduced to include DNA based vaccines especially for vaccination against respiratory diseases. These vaccines will provide better protection against infection and the possibility of a wider and safer use in third world countries. Finally, it will be interesting to follow the developments in the area of nose to brain delivery

and to ascertain whether it will prove possible to control and enhance the use of the route of delivery for treatment of diseases where rapidly achieved and high concentrations of drug in the brain are essential for an efficient therapeutic response.

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