International Journal of Pharmaceutics 379 (2009) 146–157

Contents lists available at ScienceDirect

International Journal of Pharmaceutics

journal homepage: www.elsevier.com/locate/ijpharm

Review

Nanoparticles for direct nose-to-brain delivery of drugs

Alpesh Mistry, Snjezana Stolnik, Lisbeth Illum*

Advanced Drug Delivery and Tissue Engineering Division, School of Pharmacy, University of Nottingham, Nottingham, NG7 2RD, UK

ARTICLE INFO

Article history:
Received 23 April 2009
Received in revised form 12 June 2009
Accepted 16 June 2009
Available online 23 June 2009

Keywords:
Chitosan
Drug delivery
Lectin
Nanoparticles
Olfactory
Toxicity
Trigeminal
PEG

ABSTRACT

This review aims to evaluate the evidence for the existence of a direct nose-to-brain delivery route for nanoparticles administered to the nasal cavity and transported via the olfactory epithelium and/or via the trigeminal nerves directly to the CNS. This is relevant in the field of drug delivery as well as for new developments in nanotechnology. Experiments in animal models have shown that nano-sized drug delivery systems can enhance nose-to-brain delivery of drugs compared to equivalent drug solutions formulations. Protection of the drug from degradation and/or efflux back into the nasal cavity may partly be the reason for this effect of nanoparticles. It is uncertain, however, whether drug from the nanoparticles is being released in the nasal cavity or the nanoparticles carrying the drug are transported via the olfactory system or the trigeminal nerves into the CNS where the drug is released. Furthermore, toxicity of nanoparticulate drug delivery systems in the nasal cavity and/or in the CNS has not been extensively studied and needs to be considered carefully.

Contents

1. Introduction

Diseases of the Central Nervous System (CNS) such as schizophrenia, meningitis, migraine, Parkinson's disease and Alzheimer's disease require delivery of the drug to the brain for treatment. However such transport remains problematic, especially for hydrophilic drugs and large molecular weight drugs, due to

Abbreviations: CSF, cerebrospinal fluid; %DA, % degree of deacetylation; N–B, nose-to-brain; HC-1, hypocretin-1; MW, molecular weight; PEG, polyethylene glycol; P-gp, P-glycoprotein; ROS, reactive oxygen species; TEM, transmission electron microscopy.

* Corresponding author. Tel.: +44 0115 84 685 04; fax: +44 0115 84 665 80.
E-mail address: lisbeth.illum@illumdavis.com (L. Illum).

0378-5173/$ – see front matter.
doi:10.1016/j.ijpharm.2009.06.019

the impervious nature of the endothelial membrane separating the systemic circulation and central interstitial fluid, the Blood–Brain Barrier (BBB) (Pardridge, 1999). Hence, many therapeutic agents may have been abandoned because sufficient drug levels in the brain cannot be achieved via the systemic circulation.

Macromolecular drugs such as peptides and proteins, termed 'biologics,' are too large and too hydrophilic to penetrate the BBB from the systemic circulation and would be rapidly degraded by gastrointestinal enzymes or the liver cytochromes, if taken orally. A non-invasive therapy would be desirable for patients particularly for diseases that require chronic dosing such as those related to dementia.

It has been shown in the literature from animal and human investigations, that transport of exogenous materials directly from nose-to-brain is a potential route for by-passing the BBB (Illum, 2000). This route, involves the olfactory or trigeminal nerve systems which initiate in the brain and terminate in the nasal cavity at the olfactory neuroepithelium or respiratory epithelium, respectively. They are the only externally exposed portions of the CNS and therefore represents the most direct method of non-invasive entry into the brain. However, the quantities of drug administered nasally that have been shown to be transported directly from nose-to-brain are very low, normally less than 0.1%, and hence the system is not currently used therapeutically and no product is licensed specifically via this route (Illum, 2004).

The strategy of applying drugs that are encapsulated into particulate vectors (such as synthetic nanoparticles) to the olfactory epithelium could potentially improve the direct CNS delivery of drugs—including biologics. If drugs could reach the CNS in sufficient quantity by this route, it could generate interest in previously abandoned drug compounds and enable an entirely novel approach to CNS drug delivery. Therefore, the aims of this review are to critically evaluate the evidence of nose-to-brain transport with a focus on nanoparticulate drug carriers and to suggest future strategies that may benefit progress in the field of nanomedicines. However, in order to contemplate aspects of nose-to-brain drug delivery it is necessary to have a reasonable understanding of the nasal anatomy and physiology relating to the field.

2. Nasal anatomy and physiology

This section only briefly considers the general anatomy and physiology of the nasal cavity since detailed descriptions can be found in many review papers and text books (Lledo et al., 2005; Mygind, 1979). However, in this review emphasis has been put on a discussion of the olfactory region and the trigeminal nerve systems that are both implicated in the transport of drugs from the nasal cavity to the brain.

2.1. The external nose and the nasal cavity

The main functions of the nose are olfaction, regulation of humidity and temperature of inhaled air, and removal of large particulates including microorganisms from the inhaled air. In humans, the total surface area and volume of the two sides of the nasal cavity has been measured using computed tomography (CT) scans as 150.4 cm^2 (made possible by three protrusions or 'turbinates' within the cavity) and 13.0 ml, respectively (Menache et al., 1997).

The nasal septum divides the nasal cavity along the centre into two halves open to the facial side and to the rhinopharynx, through the anterior and via the posterior nasal apertures, respectively. Each nasal cavity can be divided into three regions; the nasal vestibule, the olfactory region and the respiratory region. The olfactory epithelium is located high in the nasal cavity in man. It partly overlies the cribriform plate, a bony structure that contains many pores that allow the passage of neuronal bundles from the olfactory epithelium to pass into the CNS. Olfactory epithelium may also lie partly on the nasal septum and on the superior turbinate. It is above the normal path of the airflow which means that odorant molecules normally reach the sensitive receptors by diffusion. The act of sniffing enhances the diffusional process by increasing the airflow rate and changing it from continuous to pulsatile in nature. This behaviour increases the turbulence within the nasal cavity and therefore allows greater interaction of the inspired air with the olfactory region at the roof of the nasal cavity. The respiratory region is dominated by the large inferior turbinate, the middle turbinate and further back in the nasal cavity, the superior turbinate.

2.2. The respiratory epithelium and mucociliary clearance

The respiratory epithelium is composed of four types of cells, namely, non-ciliated and ciliated columnar cells, basal cells and goblet cells. These cells facilitate active transport processes such as the exchange of water and ions between cells and motility of cilia (where applicable). They may also serve to prevent drying of the mucosa by trapping moisture. About 15–20% of the respiratory cells are covered with a layer of long cilia, which move in a coordinated way to propel mucus towards the pharynx.

Mucus (or nasal secretion) is a complex mixture of materials consisting of approximately 95% water, 2% mucin, 1% salts, 1% of other proteins such as albumin, immunoglobulins, lysozymes and lactoferrin, and <1% lipids (Kaliner et al., 1984).

Mucus is present in two layers on the epithelium in order to facilitate mucociliary clearance. A viscous gel layer, the 'mucus blanket' (Fig. 1c; 'gel layer', 2–4 μm thickness) floats on the serous fluid layer (Fig. 1e 'sol layer', 3–5 μm thickness). The viscous gel layer is moved along by the hook shaped cilia termini during the energy-dependent 'effective stroke' phase of the ciliary motion (Fig. 1a). Cilia are up to 7 μm in length when fully extended but can fold to half this length during the recovery stroke where the hook terminus detaches from the gel layer and moves immersed in the sol layer in the opposite direction to the gel layer movement (Fig. 1b). The cilia beat with a frequency of 1000 strokes per min. Hence the mucus moves only in one direction from the anterior to the posterior part of the nasal cavity to the nasopharynx. Therefore, particles applied to the nasal respiratory mucosa will be transported on the mucus to the back of the throat with a speed of 5 mm per min (Lenaerts and Gurny, 1990; Mygind, 1979).

2.3. Olfactory epithelium and neuronal supply to the nasal cavity

The olfactory epithelial layer predominantly contains three cell types: the olfactory neural cells, the sustentacular (also known as supporting) cells and the basal cells. Basal cells are progenitor cells (of supporting cells) that also provide mechanical support via anchorage to other cells.

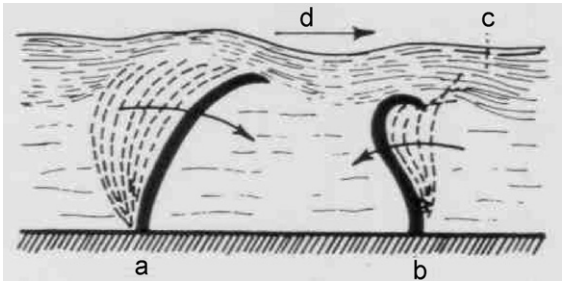

Fig. 1. The relationship between ciliary motion and mucus layer composition that allows mucociliary clearance. (a) effective stroke, (b) recovery stroke, (c) gel layer, (d) direction of gel layer movement, and (e) sol layer. Adapted from Proetz (1953).

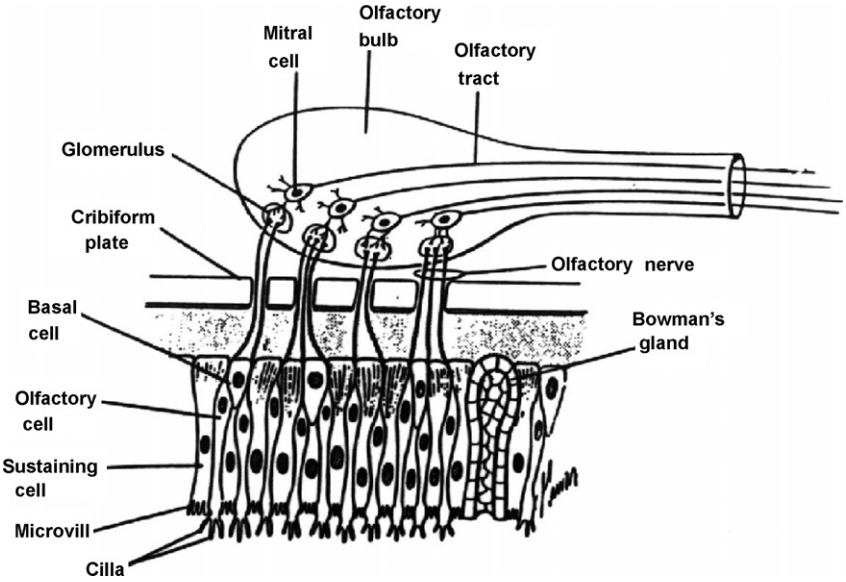

Fig. 2. Diagram of the olfactory area showing the olfactory epithelium, bulb and tract. From Adams et al. (1989).

The olfactory neural cells or the axons are un-myelinated and interspaced between the supporting cells (Fig. 2). They originate at the olfactory bulb in the CNS and terminate at the apical surface of the olfactory epithelium.

The olfactory knob (or vesicle) protrudes out from and above the apical surface of the olfactory epithelium (Fig. 3). Approximately 10–23 cilia project from the basal bodies of the knob, each of length up to 200 μm. The cilia contain chemical detectors that, once activated by odorants, initiate depolarisation of the olfactory axon by either direct ion-gated channels or cAMP operated ion-channels (Gartner and Hiatt, 2000). The cilia entangle with the thick brush-border of microvilli of the supporting cells at the air/mucus/tissue interface (Fig. 3). The cilia are non-motile in the olfactory region (in contrast to respiratory tissue) since they lack the dynein arms which contain the Mg^{2+}-ATPase that generates the force for ciliary motility (Moran et al., 1982).

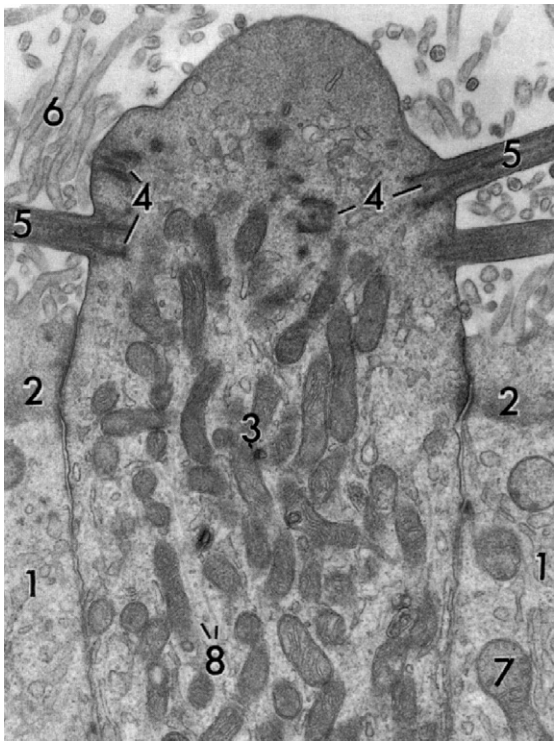

Fig. 3. Electron microscope images of cat olfactory knob. (1) Neighbouring supporting cell; (2) tight junction; (3) mitochondrion in olfactory knob; (4) basal bodies; (5) olfactory cilia; (6) microvilli; (7) mitochondrion in supporting cell; (8) microtubules. From Rhodin (1974).

The *lamina propria* of the olfactory epithelium, which is located beneath the epithelial layer(s), contains the blood supply, mucus secreting acinar glands (Bowman's glands), nasal lymphatics, and a neuronal supply that consists of olfactory axon bundles, autonomic nerve fibres and the maxillary branch of the trigeminal nerve (Brand, 2006; Brodbelt and Stoodley, 2007; Mygind, 1979; Tucker, 1971). Bowman's glands are under the control of the parasympathetic nervous system. These acinar-type glands produce nasal secretions in the *lamina propria* and secrete them through a narrow tube-like opening into the luminal space.

In the *lamina propria* the olfactory neurones taper together and are ensheathed by glial cells (or Schwann cells). These processes are called *filia olfactoria*. De Lorenzo (1960) has described the *filia olfactoria* in detail. *Filia olfactoria* are unique features in the mammalian body in that around twenty axons are partitioned by the Schwann cell into fascicles (Fig. 4). In this way a single Schwann cell may ensheathe around a hundred or so axons. This feature allows 10–15 nm sized spaces between axons that act as ionic reservoirs for action potential propagation. Hence, perineuronal transport of molecules to the olfactory bulbs is limited by the size of these spaces. Mesaxons are pores in the *filia olfactoria* structure that allow passage of extracellular fluid into the neuronal bundle structure.

The average diameter, by electron microscopy, of olfactory axons in 2-month-old rabbits is ~200 nm, however, many of the axons have diameters of <100 nm (De Lorenzo, 1960). Theoretically therefore transcellular transport of up to 200 nm diameter particles is possible in these animals. Other species show similar olfactory axonal diameters, for example, the African Clawed frog, various bird species and humans have diameters of 198 ± 93, 210–260 and 100–700 nm, respectively (Burd, 1991; Matsuzaki, 1995; Morrison and Costanzo, 1992).

The Schwann-sheathed axonal bundles then pass through the *lamina propria* and into the porous structure of the cribriform plate. Around 1500 sensory neurones synapse on one mitral cell in the glomeruli of the olfactory bulb. The mitral and tufted cells are

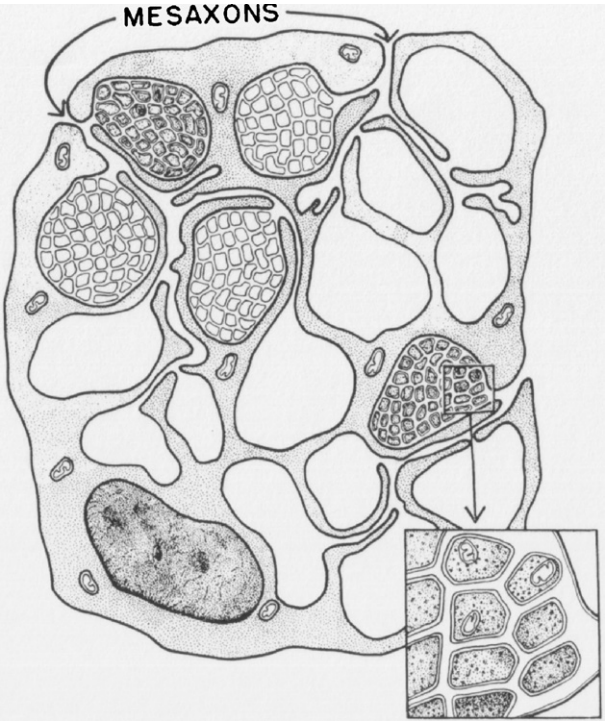

Fig. 4. Schematic representation of *fila olfactoria*. The arrangement of neurones is unusual in that many (~20) neurones are isolated into fascicles. Here five such fascicles are ensheathed by a single Schwann Cell. In addition, the cavities in the structure (C) provide reservoirs for ions. This enables ion exchange between the axoplasm and the extracellular fluid. Mesaxons are pores in the structure that allow continuity between the extracellular fluid and the *filia olfactoria*. Adapted from De Lorenzo (1960).

branched; they project one dendrite to each glomerulus. These neurones then pass through to the olfactory tubercle. From there, the third-order neuronal projections pass to the amygdala, prepyriform cortex, the anterior olfactory nucleus and the entorhinal cortex as well as the hippocampus, hypothalamus and thalamus (Lledo et al., 2005). It is envisaged based on findings for large proteins (Shipley, 1985) and studies on nanoparticles (Oberdörster et al., 2004) that nanoparticles of sufficiently small size could potentially be transported via axons through the olfactory bulb into the olfactory cortex and from there to the caudal pole of the cerebral hemisphere and into the cerebrum and the cerebellum. Hence, these are all potential delivery sites for the nose-to-brain drug transport route via olfactory epithelium.

The trigeminal nerve is the largest of the cranial nerves and, amongst other functions, enables sensory perception in the nasal cavity. It has three major branches namely: the ophthalmic nerve, maxillary nerve and mandibular nerve. The ophthalmic and maxillary nerves only have a sensory function whereas the mandibular nerve has both sensory and motor functions. The three branches meet at the trigeminal ganglion which contains the cell bodies of these sensory nerve fibres. Afferent neurones synapse at the trigeminal ganglion to form a single incoming nerve that enters the brainstem at the level of the pons.

The ophthalmic and maxillary branches of the trigeminal nerve are important for nose-to-brain drug delivery since neurones from the branches pass directly through the nasal mucosa. In fact, these neurones have been proven to deliver the neurotrophic factor, IGF-1 (MW 7.65 kDa), to the brainstem and spinal cord areas in the *in vivo* rat model (Thorne et al., 2004). Hence, in contrast to rostral entry of drug via the olfactory pathway, the trigeminal nerve was shown to enhance nose-to-brain delivery to caudal brain areas.

2.4. Junctional complexes

Junctional complexes are cell-to-cell contact areas found between both respiratory and olfactory epithelial cells in the nasal cavity. There are three different types of these complexes; *macular adherens* (or desmosomes), *zonula adherens* and *zonula occludens* (tight junctions [Fig. 3]) (Gumbiner, 1987; Van Itallie and Anderson, 2006); The *zonula occludens* are located closest to the luminal space above the *zonula adherens* with the *macular adherens* situated basolaterally in relation to the other two complexes. Thus, transport of drugs between cells (paracellular transport) is largely determined by the integrity of these (and in particular the *zonula occludens*) complexes for intact epithelia.

The hydrophilic channel between epithelia, the tight junctions, are generally impermeable to molecules with a hydrodynamic radius greater than 4–8 Å. This is the normal diameter of a closed tight junction (depending on the 'leakiness' of the epithelium) which restricts the movement of molecules larger than this to pass between neighbouring cells (McMartin et al., 1987). It was found by Miyamoto et al. (2001) that in the nasal epithelia, the largest molecular weight drug that was transported paracellularly (albeit at very low amounts) without the addition of an absorption enhancer was about 50 kDa. Even when tight junctions are opened, by application of certain absorption enhancers such as poly-L-arginine, they reach a maximum of about 15 nm in diameter and hence are not expected to allow molecules larger than 150 kDa to pass through in significant quantities (Miyamoto et al., 2001; Costantino et al., 2007). Hence, generally nanoparticles used in the literature are too large for this route to achieve feasible transport for drug delivery purposes.

3. Cellular mechanisms for transmucosal drug delivery

Nanoparticles (when larger than about 20 nm) are thought to pass transcellularly (apical to basolateral transport through epithelial cell) in nose-to-brain drug delivery (Mistry et al., 2009). The transcellular route of cell transport is less well characterised than the paracellular route (Miaczynska and Stenmark, 2008). Novel spectroscopy and microscopy techniques such as electron energy loss spectroscopy and energy filtering transmission electron microscopy have recently provided new insights into endocytosis and the cellular mechanism responsible for the transcellular transport of particles (Rothen-Rutishauser et al., 2006).

Endocytosis has been categorised by a number of different molecular mechanisms including macropinocytosis, clathrin-mediated, clathrin-independent, caveolin-mediated, caveolin-independent and phagocytosis (Conner and Schmid, 2003). Macropinocytosis is an endocytic mechanism where the action of actin filaments gives rise to curved 'ruffles' on the cell surface. Sealing of the aperture into discrete vacuoles forms the macropinosome (0.5–5 μm diameter) which efficiently takes up extracellular fluid into the cell (Xiang et al., 2006). Considerable volumes of dissolved molecules and suspended particles can be taken up in this way. Macropinocytosis is generally thought of as a constitutive process by which the cell can sample the extracellular environment and is not believed to be initiated by receptor activation at the cell surface (Xiang et al., 2006).

Receptor-meditated endocytosis is a term used to describe a group of endocytic mechanisms where the 'cargo' is thought to stimulate the endocytic event by complementing a receptor on the cell membrane. Receptor-mediated endocytosis can involve either clathrin-dependent or independent mechanisms. The clathrin protein is endogenously expressed within mammalian cells in the form of a heavy chain and a light chain. The fusion of these chains results in the formation of a triskelion structure, a 3D array of which produces a clathrin coat (Edeling et al., 2006).

Recruitment of the coat to the cytosolic cell membrane gives rise to regions called 'coated pits' during the initial stages of receptor-mediated endocytosis. Invagination of the pit results in a clathrin-coated vesicle that is taken into the cytosol. These vesicles can appear in various shapes and sizes but are generally believed to be <150 nm in diameter (Xiang et al., 2006). Phagocytosis is a clathrin-independent receptor-mediated uptake of exogenous materials by specialised phagocytic cells such as macrophages. Relatively large (>1 μm) patches of membrane are internalised (Mayor and Pagano, 2007) and the exogenous material engulfed into lysosomes either for destruction or antigen presentation. Hence, phagocytic cells act to destroy materials that may be harmful to other cells and also alert immune system cells to their presence.

Phagocytic cells make up a minority of the total cell population in the nasal cavity and therefore are not thought to contribute to cellular uptake in therapeutic concentrations.

Overall, it is not yet fully understood how substrates such as nanoparticles initiate the process of cellular internalisation. Clathrin-dependent endocytosis is currently the best characterised endocytic mechanism, yet there is still debate over exactly how the exogenous particles initiate the event (Sorkin, 2004).

For example, one fundamental study has shown that basic parameters such as particle size strongly influence the initiation of certain endocytic mechanisms over others (Rejman et al., 2004). These authors incubated fluorescently labelled polystyrene nanoparticles of 50, 100, 200, 500 and 1000 nm diameter with murine melanoma cells (B16–F10). The cells were treated with selective endocytic inhibitors to reveal the pathways used to internalise the nanoparticles. Confocal microscopy revealed that <200 nm diameter nanoparticles were involved with clathrin-coated pits. However, caveolae-mediated endocytosis became more apparent as the nanoparticles increased in size (200–1000 nm). Moreover, 50–100 nm diameter particles were more rapidly internalised into cells than 200 nm nanoparticles; the mechanism for this was unclear.

Another factor that influences the internalisation pathway of particles is their surface charge. Harush-Frenkel et al. (2008) produced anionic 90 nm diameter PEG–PLA nanoparticles and a cationic version which incorporated the cationic lipid stearylamine. They incubated these particles separately with MDCK (Canine Kidney Epithelial) cells and used confocal microscopy, immunofluorescence and Western blotting to determine that both types of particles entered the cell via the clathrin-mediated endocytic pathway which is in agreement with Rejman et al. (2004). However, the cationic nanoparticles avoided the downstream lysosomal pathway as opposed to the anionic particles.

Other factors that influence endocytic trafficking could include cell type and concentration of particles applied to the cells (Jones, 2008). Moreover, a number of endocytic pathways simultaneously translocate particles into cells, and therefore, cellular regulatory mechanisms may compensate for deliberate inhibition of certain endocytic pathways by upregulating other endocytic pathways; this leads to difficulty in data interpretation.

With respect to nose-to-brain administration of different nano-sized particles to the nasal mucosa it is clear that this may lead to cell internalisation by different endocytic pathways. The fate of nanoparticles in the endocytic pathway is dependent on the size and surface characteristics of the particles which could be used as a means of improving their cellular uptake into olfactory epithelial cells.

Finally, the current unpredictability of endocytic trafficking of nanoparticles strengthens the argument to deliver drug molecules using a single platform technology whose cellular internalisation and distribution characteristics are well defined in olfactory axons.

4. Direct transport of drugs in solution from nose-to-brain

Investigations of the transport pathways of drugs from the nasal cavity to the CNS have been a result of the observations made from nasal administration of therapeutic drugs, from viral particles and heavy metal poisonings of the brain. It has been found in animal models that increasing the drug hydrophilicity (Sakane et al., 1991), molecular weight (above 20 kDa (Sakane et al., 1995)) and degree of ionisation (Sakane et al., 1994) can reduce drug transport into the CNS after *i.n.* administration. In addition, small molecular weight drugs are also affected by the active efflux transporter pumps at the apical membrane surface (P-gp) or enzymatic degradation in the olfactory epithelium (Chou and Donovan, 1997; Dahlin et al., 2001). Therefore, transport of a drug directly into the CSF, as a measure for CNS delivery, is determined by a combination of molecular and biological properties of the drug which are at this stage difficult to predict. Finally, a number of studies have shown that CNS bioavailability of small molecular weight drugs after *i.n.* instillation is very low (typically less than 0.12% of administered dose for sulphonamides (Sakane et al., 1991), dopamine (Dahlin et al., 2001) and morphine (Westin et al., 2005)). Detailed analyses of direct nose-to-brain delivery of drug solutions can be found elsewhere (Illum, 2000, 2004; Merkus and van den Berg, 2007).

Relatively fewer studies have been performed on human subjects than animal models. Human volunteer studies do not normally report absolute measurements of drug in the CSF or brain tissues since it would be unethical to do so. Attempts have been made to evaluate the direct nose-to-brain behavioural effects of various endogenous hormones (vasopressin (Pietrowsky et al., 1996a), cholecystokinin (Pietrowsky et al., 1996b), angitensin (Derad et al., 1998) and insulin (Kern et al., 1999; Benedict et al., 2008)). However, the transport of these molecules via the olfactory system has not been conclusively proven since it could not been excluded that they innervated the nasal neuroepithelia by receptor–ligand interactions.

Born et al. (2002) studied the nasal administration of melanocortin 4–10 (MSH/ACTH 4–10), vasopressin and insulin in 36 human volunteers in a placebo controlled study with sampling of CSF and blood for up to 80 min after administration. Due to lack of a parenteral (intravenous) control the results obtained should be viewed with some caution, but nevertheless the fact that no increase in serum levels was detected after nasal administration of MSH/ACTH 4–10 and insulin concurrent with an increase the concentration of these drugs in the CSF strongly indicated that these two drugs reached the CSF via direct nose-to-brain delivery. Merkus et al. (2003) presented results from a study in 28 postoperative neurology patients (only 8 patients completed the study) who received nasal and intravenous administrations of either hydroxycobalamin (5 patients) or melatonin (3 patients). Samples of blood and CSF were withdrawn for up to 180 min. For the lipophilic ($\log P \sim 1.65$) drug melatonin there was no difference in CSF appearance whether drug was given nasally or intravenously, most like due to the drug entering the CSF via the bloodstream after nasal application. For hydroxycobalamin the authors also suggested that no direct nose-to-brain transport had taken place based on the calculation of the $(AUC_{CSF}\ IN/AUC_{plasma}\ IN)/(AUC_{CSF}\ IV/AUC_{plasma}\ IV)$ ratio as 1.0. However, as discussed by Illum (2004) calculating these ratios on an individual patient to patient basis and not on mean AUC values for all patients as done in the Merkus paper, showed that the ratio was 1.65, indicating a distinct direct nose-to-brain transport in these patients.

Recently a study has shown that HC-1, which has receptors deep inside the CNS and is therefore less likely to innervate the olfactory neuroepithelium when administered nasally, can relieve cataplexic narcolepsy (Baier et al., 2008). This is a promising study that shows that direct nose-to-brain drug delivery may be clinically

successful in the future. Nevertheless, further studies could combine behavioural investigations with radiolabelled drugs so that pharmacological effects and location of radioactivity may be correlated to provide stronger evidence of direct nose-to-brain drug delivery in humans.

5. Nose-to-brain transport of nano-sized vectors

Nanoparticles may offer an improvement to nose-to-brain drug delivery since they are able to protect the encapsulated drug from biological and/or chemical degradation, and extracellular transport by P-gp efflux proteins. This would increase CNS availability of the drug. A high relative surface area means that these vectors will release drug faster than larger equivalents; a property desirable where acute management of pain is required. Their small diameter potentially allows nanoparticles to be transported transcellularly through olfactory neurones to the brain via the various endocytic pathways of sustentacular or neuronal cells in the olfactory membrane, as described above. Surface modification of the nanoparticles could achieve targeted CNS delivery of a number of different drugs using the same 'platform' delivery system which has known and well characterised biophysical properties and mechanism(s) of transit into the CNS.

5.1. Transport and toxicity of elemental nanoparticles

To date, very few studies have specifically examined the direct transfer of particles from the nasal cavity to the brain. Mostly basic particulate materials such as manganese oxide (Elder et al., 2006), gold (De Lorenzo, 1970), carbon-13 (Oberdörster et al., 2004), iridium-192 (Semmler et al., 2004), iron (II) oxide (Wang et al., 2007) and titanium dioxide (Wang et al., 2008a), have been used. For instance, to demonstrate the transport of nanoparticles within the olfactory region, rats (200–250 g body mass) were allowed to inhale poorly soluble salts of manganese (MnO, Mn_2O_3) applied as an aerosol (Elder et al., 2006). The nanoparticles had a diameter of 30 nm. Manganese was detected, after 6 and 12 days of inhalation exposure (these were repeated exposures of 6 h/day, 5 days/week), in the olfactory bulbs and also in deep brain structures such as the cortex and cerebellum by using graphite absorption spectroscopy of microdissected brain structures (olfactory bulbs, striatum, trigeminal ganglions, midbrain, frontal cortex, and cerebellum). The manganese salts used in this study were poorly soluble and therefore the manganese was thought to have remained in the particulate form when transported into the brain. Unfortunately, images of the (30 nm) nanoparticles in these brain regions were not shown in the publication therefore strong evidence of actual uptake of the manganese salt in particulate form was not presented; it is conceivable that the salt form could change *in vivo* so that a soluble manganese salt (e.g. $MnCl_2$) is formed.

In an older study, De Lorenzo (1970) visualised exogenous particulate matter (50 nm colloidal gold particles) within the olfactory neurones by TEM. The gold colloid was administered nasally to rats and suggested to enter the receptor cells by an endocytic process into the axoplasm within 30 min. The colloidal particles moved along the axon, possibly by a mechanism related to microtubules in the cytoplasm. The velocity of this movement was estimated to be 2.5 mm/h (De Lorenzo, 1970). This is in line with observations made of poliomyelitis virus in rhesus monkey sciatic nerve (2.4 mm/h) (Bodian and Howe, 1941). Within 60 min of application to the nasal cavity the gold particles were seen in the *filia olfactoria* and olfactory glomerulus having crossed the (first-order) synapse to reach the mitral cells. At this point (in the olfactory bulb) the particles were mostly located within mitochondria. The mechanism for such an accumulation is unclear, but it is evident that translocation of nanoparticles into the mitochondria can lead to cellular toxicity (Xia et al., 2008). Furthermore, the particles did not appear to travel extra-axonally which supports the idea that nanoparticles are too large to be transported paracellularly (Miyamoto et al., 2001).

In another experiment, 14-week-old rats (mean body mass 284 g) were exposed twice to (insoluble) ~36 nm in diameter elemental ^{13}C nanoparticles (150–170 $\mu g/m^3$). The exposure took place in an inhalation chamber for a total of 6 h (Oberdörster et al., 2004). Exposing the rats to the nanoparticles, applying an inhalation chamber, is thought to allow nanoparticles access to the rat nasal cavity, since the animals are obligate nasal breathers. The results showed a significant and persistent increase of ^{13}C of 0.35–0.43 $\mu g/g$ in the olfactory bulbs from day 1 to day 7 following treatment. However, a deeper brain penetration of these particles into the cerebellum and cerebrum was not conclusively demonstrated, since a significant increase in radioactivity was not consistently observed during the full duration of the experiment. Repeated exposures might have increased the concentration of ^{13}C nanoparticles in the cerebrum and cerebellum.

Low levels of 20 nm sized iridium-192 nanoparticles (insoluble) have also been observed over a 6-month period in the brain after inhalation of a single dose of particles via an intratracheal tube (Semmler et al., 2004). A peak number of particles were found in the rat brains 7 days after a 60–90 min single exposure. This rate of transfer appears to be much slower than the movement of 50 nm gold particles from the nasal cavity to the olfactory bulbs in rabbits as described earlier (De Lorenzo, 1970). The differences between the rates of transport could have been due to the different time points at which samples were taken in each case and the administration site.

A recent study administering a dose of 40 mg/kg Fe_2O_3 nanoparticles (280 ± 80 nm diameter) nasally to 4-week-old male mice (20–22 g), demonstrated a deep brain penetration of the metal oxide and its potential to disrupt the cellular morphology in the hippocampus (Wang et al., 2007). The olfactory bulbs and brain stems were found to contain a larger amount of iron than the control group 14 days after exposure. The hippocampus of the Fe-exposed group was also histologically dissimilar to that of the control group with 'fatty degeneration' features found in the hippocampus region. It was suggested that the Fe_2O_3 had been transported directly from the nasal cavity to the brain. Unfortunately, an *i.v.* control group, was not included in the study and hence the brain uptake may have been due to systemic absorption of Fe from the nasal cavity and subsequent translocation to the deep brain regions via receptor-mediated uptake at the BBB.

The same group later nasally instilled 500 μg of 71.4 ± 23.5 nm TiO_2 nanoparticles in mice (Wang et al., 2008a). At day 30 after nasal application the mice serum biomarkers (enzyme activity and cytokine levels) were found to be normal compared to control mice. This implied that the TiO_2 nanoparticles did not pass into the systemic circulation in significant quantities; however, absolute serum concentrations were not measured. The TiO_2 nanoparticles were suggested to have passed into the olfactory neurones and further into the hippocampus via the olfactory bulb and olfactory cortex since at day 30 post-changes to the Nissl bodies and nuclei in cells of the olfactory bulbs and hippocampi were demonstrated and a number of brain enzymes and cytokine levels were elevated. However, Ti-containing nanoparticles were not shown in the electron microscope images. Therefore, the exact mechanisms of the damaging affects of TiO_2 still need to be elucidated.

The ability to visualise actual nanoparticles in subcellular organelles is an important requirement in these studies, because this allows correlation of cellular damage with size and number of nanoparticles *in situ*. It is thought that nanoparticles may damage cells by gaining access to cell organelles (such as mitochondria) that larger particles may not (Xia et al., 2008).

5.2. Transport and efficacy of nanoparticles and nanoemulsions

The intranasal application of elemental particles has demonstrated the potential for direct nose-to-brain transport of particulate matter. These studies were mostly carried out in relation to an evaluation of the toxicity to the CNS of environmental nanoparticles/pollutants. From a drug delivery perspective, application of nanoparticles composed of polymers (which are typically used in drug delivery) have shown statistically greater ability, than a simple formulation of the drug, to deliver model drugs such as nimodipine to the olfactory bulb (Zhang et al., 2006) or to enhance the pharmacological activity of morphine (Betbeder et al., 2000), when these small molecules were applied intranasally in combination with nanoparticles.

Hence, significant enhancement of nimodipine delivery to the CSF and olfactory bulb was reported when intranasally applied to rats in methoxy-PEG–PLA (MPEG–PLA) nanoparticles (76.5 nm diameter) compared to drug in solution (Zhang et al., 2004). There is a possibility that the nanoparticles containing drug were endocytosed by the olfactory epithelial cells and the drug released, however, it is also highly likely that the drug would have been released from the nanoparticles present in the mucous layer from where it would be absorbed paracellularly or transcellularly in the epithelium.

Nasally applied [^{3}H]morphine increased analgesia in mice when administered in conjunction with 60 nm sized maltodextrin nanoparticles (Biovectors^{TM}) compared to a [^{3}H]morphine solution (Betbeder et al., 2000). The effect could not be attributed to increased levels of [^{3}H]morphine in the blood, with subsequent transport into the brain via the BBB, since an *s.c.* dose of morphine produced higher plasma levels but lower analgesia than the *i.n.* dose of the morphine-Biovectors^{TM} system. Also, *i.n.* administered morphine-Biovectors^{TM} produced similar plasma levels as *i.n.* morphine solution and hence did not indicate increased transport of drug across the BBB from the blood stream. It was suggested by the authors, that morphine was transported via a direct nose-to-brain pathway. The analgesic effect was reversed by naloxone, an opioid antagonist, confirming that the analgesia was opiod-induced. Although this study indicates that morphine may have been transferred by the direct nose-to-brain route, the exact mechanism of the increased analgesia was uncertain. For example, the morphine did not adsorb to the nanoparticles *in vitro* and the analgesic effect was not enhanced by the addition of an absorption enhancer to the solution formulation. The fate of the nanoparticles *per se* was not investigated in this study, and hence it is not possible to deduct their role in the potential nose-to-brain transport of the morphine.

In general, there is a lack of literature results confirming actual transmucosal movement of particles in the olfactory or in the respiratory epithelia. In general, most studies are limited to demonstrate an improvement in drug transport and effect of the drug when administered in a nanoparticle formulation as compared to the 'simple' drug solution, but no proof of the actual mechanism of improvement of transport of the drug is given (Illum, 2006; Chen et al., 2008; Dalpiaz et al., 2008). In fact, the mechanisms of transport of the nanoparticles across the epithelial layer are not yet fully understood. Huang and Donovan (1996) studied the transport of polystyrene (PS) nanoparticles (10–500 nm in diameter) across excised rabbit nasal respiratory epithelium using fluorescence spectroscopy and confocal microscopy. They found that 10 nm carboxylated PS particles and 200 nm amine-modified PS particles were transported both paracellularly and transcellularly, but only in miniscule quantities. 100 nm and 500 nm carboxyl-modified PS could only access the transcellular route. The authors' suggested that nanoparticles with relative hydrophilic surfaces (amine- and carboxylate-modified) could pass through the aqueous paracellular route; whereas hydrophobic carboxyl-modified PS appeared to mainly be transported via the transcellular pathway because of restricted access to the hydrophilic paracellular channels. However it seems unlikely that paracellular transport of 200 nm PS is possible, due to the size restriction imposed by the apically situated tight junctions, as discussed above. Des Rieux et al. (2005) investigated the transport of carboxylated and aminated (200 and 500 nm diameter) polystyrene nanoparticles (FluoSpheres) across monolayers of Caco-2 cells and co-cultures of Caco-2 and human Raji B lymphocytes. The study showed a dependence of the transport on the size and surface characteristics of the nanoparticles and the type of cell culture used. In general, very low amounts of particles were transported through either of the cell cultures, the smaller particles were transported to a higher degree than the larger particles, and aminated particles were transported through co-culture systems better than carboxylated particles. Interestingly, in all instances particles were transported to a much higher degree through co-cultured cell monolayers that included M-like cells which are known to transport particulate matter. Such cells are present in the nasal cavity.

Almeida et al. (1993) evaluated in an *in vivo* study the transport of carboxylated polystyrene particles (510 nm in diameter) applied to the nasal mucosa into the systemic circulation in rats and rabbits. 10 min after application, 0.96% and 1.0–2.9% of the particles were found in the bloodstream of the rats and the rabbits, respectively. The authors however, did not provide a discussion to the possible mode of transport of the particles. Brooking et al. (2001) demonstrated transmucosal transport of 100 nm PS particles into the systemic circulation after *i.n.* administration in rats. The blood concentration was 2% of the administered dose after 3 h. This was attributed to the passage of nanoparticles through non-olfactory epithelium via antigen sampling M-cells. In the same study negligible amounts of 100 nm PS were determined in whole brain homogenate 3 h after administration, although microdissection of the brain was not performed to ascertain their location.

However, these positive examples of transmucosal PS delivery following *i.n.* application are opposed by other studies which suggest that PS latex is not transported across an epithelial cell monolayer. For example, Ma and Lim (2003) found that chitosan nanoparticles (~500 nm in diameter) were internalized into Caco-2 cells in monolayers when applied apically. The uptake was time and concentration dependent. However, no nanoparticles were found to be transported across the monolayer and when insulin was associated with the nanoparticles, no insulin was detected at the basolateral side after 4 h incubation, although present in the cells. In a similar study, Sadeghi et al. (2008) evaluated the transport of insulin across Caco-2 cell monolayers when formulated in 200 nm sized nanoparticles made from chitosan, and derivatised chitosan. Interestingly, the amount of insulin transported was highest when administered in combination with the chitosan, or chitosan derivative, as soluble polymers, as compared to the nanoparticle systems made from the same polymers. The authors suggested that the nanoparticles that were transported crossed the monolayer by the transcellular route. However, there was no evidence provided for such an explanation.

The potential role of the mucus layer in the transport of nanoparticles in epithelial tissues can be illustrated by the study of Behrens et al. (2002). They evaluated the uptake of 213 nm PS nanoparticles into mucus producing cultured MTX cells. Analysis of cells following 120 min incubation with nanoparticles (using fluorescence spectroscopy) showed a 2-fold increase in the amount of internalised nanoparticles when the mucus layer of the cells was removed prior to the experiment. This demonstrated that mucus can be an effective barrier against transmucosal movement of nanoparticles.

5.3. Formulation strategies for enhancing direct nose-to-brain drug transport

As previously discussed, using a specific nanoparticle system as a platform technology opens a possibility to efficiently deliver a number of different drugs. One approach would be to surface modify nanoparticles, so that the drug delivery properties of the formulation are determined by the interaction of the surface coating with the biological system and not that of the biophysical properties of the drug molecule itself. This strategy could potentially standardise the utility of this drug delivery route so that it would be more predictable. This section highlights the key studies concerning chitosan, PEG and lectins in this capacity, as well as suggesting novel approaches for future consideration.

5.3.1. Chitosan surface modification

In a study in human volunteers, the ability of a 1% chitosan glutamate (MW 200–600 kDa, 75–90% DA) in saline–acetate buffer (pH 4.5) to target the olfactory region when administered as nasal drops or nasal sprays was compared (Charlton et al., 2007a). Nasal drops were administered in the supine position with the head tilted back so that the formulation could drain to the olfactory epithelium at the roof of the nasal cavity. The nasal spray was administered in the upright position. It was found by nasendoscopy that nasal drops delivered the chitosan formulation over the olfactory mucosa better than the spray formulation. In addition, the olfactory residence time of chitosan at the olfactory epithelium was increased as compared to the saline–acetate buffer (pH 4.5) control, from 1.33 to 12.6 min. Hence, a chitosan-based nasal drop formulation could be a useful approach in the future. Although no mucociliary clearance mechanism exists on the olfactory epithelium mucus will be cleared due to a solvent drag effect and due to the upright posture of humans.

Chitosan has also been widely documented to affect the permeability of epithelial membrane by its interaction with the junctional complexes between epithelial cells. The chitosan can transiently open the tight junctions and consequently allow a paracellular passage of materials through the epithelial barrier. This effect is attributed to chitosan's ability to translocate the junctional proteins, ZO-1 and claudins, from the cell membrane to the cytosol; and functionally alter the tight junction protein, ZO-1, by changes in protein kinase Cα (PKCα) activity (Smith et al., 2004, 2005).

Nanoparticles produced from cross-linked chitosan have been used in a number of studies, including a recent report by Wang et al. (2008b). They produced chitosan nanoparticles with a diameter in the range of 265–274 nm and a zeta potential of +24.8 to +26.2 mV (loaded with β-cyclodextrin complexed estradiol) using an ionic gelation method. The nanoparticle suspensions were administered to rats by the *i.n.* or *i.v.* routes. Significantly increased amounts of drug (expressed as AUC) were measured in the CSF after *i.n.* application as compared to *i.v*. administration, for a similar plasma concentration, indicating that estradiol was transported from the nasal cavity directly to the CSF. The CSF AUC for an *i.n.* estradiol solution was previously found to be similar to that after *i.v*. administration (Wang et al., 2006). The study suggests that, following nasal application, the chitosan nanoparticle formulation can achieve enhanced direct nose-to-brain transport as compared to a simple estradiol solution.

Recently, several studies in rodents have shown that direct nose-to-brain transport of small molecular weight drugs is enhanced by application in a nanoemulsion, and surface modified nanoemulsion formulation. For example, risperidone has a greater efficacy for direct nose-to-brain drug delivery when applied as a chitosan-coated nanoemulsion formulation compared to nanoemulsion alone and to a simple solution formulation (Kumar et al., 2008a). A risperidone nanoemulsion (globule diameter 15.5 ± 0.92 nm; zeta potential -12.0 ± 1.43 mV) and a chitosan coated risperidone nanoemulsion radiolabelled with ^{99m}Tc were traced *in vivo*. An equivalent dose of risperidone was administered intravenously and nasally in rats for all formulations. The results showed that all risperidone receiving groups significantly increased hind limb retraction times (from 2 ± 3 to 29 ± 5 s) and significantly reduced locomotor activity (from 223 ± 9 to 52 ± 5 counts) compared to saline. A higher amount of radioactivity was measured systemically than centrally as found by both γ-scintillation and γ-scintigraphy. It was shown that the highest concentrations of risperidone in the brain were obtained with the chitosan modified mucoadhesive nanoemulsion formulation. The concentrations were higher than for the simple nanoemulsion formulation and the risperidone solution. It was found that 78%, 57% and 62%, respectively of the risperidone delivered to the brain was transported via direct nose-to-brain delivery. The mechanism of transport was not discussed in detail.

Similar studies were performed by the same group for olanzapine (Kumar et al., 2008b) and tacrine (Jogani et al., 2008) showing that the mucoadhesive nanoemulsion system approach could be successfully translated into a platform technology. However it should be noted that, for both drugs, delivery from the nasal cavity to the blood compartment was greater than that to the brain. A sympathomimetic additive could be supplemented to the formulation in order to attempt a reduction of the systemic absorption, as discussed later.

In our own work, we studied the effect of chitosan coating on the *in vitro* uptake and transport of 100 nm polystyrene particles over porcine olfactory epithelium mounted in a Franz Diffusion cell (Mistry, 2008) and in an *in vivo* murine animal model (Mistry et al., 2009). We found, in both models, that surface modification of PS with chitosan retained greater numbers of nanoparticles in the mucus layer compared to unmodified equivalents. We also observed that increasing the cationic charge on the chitosan modified particles, by reducing the pH of the buffer from pH 6.0 to 4.5 in the porcine model, increased particle association with the mucus from 10 ± 3% to 39 ± 4% of the administered dose. This demonstrated that the mucoadhesion was primarily controlled by electrostatic interactions between mucus and chitosan-coated nanoparticles. The *in vivo* studies conducted in mice illustrated that carboxylated PS nanoparticles and PS nanoparticles coated with polysorbate 80 (PEG containing) were internalized by the olfactory cells to a higher degree than chitosan coated counterparts. The accumulation of chitosan coated latex in mucus was seen but in no circumstances were the nanoparticles found in the olfactory bulbs or further into the brain after 4 days repeated *i.n.* administration (Mistry et al., 2009).

The enhanced direct nose-to-brain drug delivery effect of chitosan formulations is suggested to be attributable to a combination of: (i) passive targeting ability of chitosan by mucoadhesion resulting in increased residence time of the formulation over the olfactory region; and (ii) increased permeability of the nasal epithelia to drug due to tight junction opening between apical cells. The latter may be due to excess free chitosan in solution and/or to a lesser degree surface coated or surface trapped chitosan. Furthermore, nanoemulsion formulation strategies may offer a safer alternative to nose-to-brain delivery of polymeric nanoparticles. One may speculate that a nanoemulsion formulation is less likely to initiate oxidative stress through mitochondrial disruption since it does not contain solid surfaces. Such solid surfaces may mechanically disrupt mitochondrial membranes and cristae as may be the case for polymeric nanoparticles. This has been discussed in detail by Oberdörster et al. (2005) although more research is needed in this area.

5.3.2. PEG surface modification

It was shown in our recent *in vivo* mouse studies that a PEG surface modification of PS nanoparticle by adsorption of polysor-

bate 80–100 nm PS latex did not significantly change the number of nanoparticles transported into the olfactory epithelial cells compared to an unmodified control (Mistry et al., 2009). Therefore, polysorbate 80 (MW 1.3 kDa) did most likely not alter the 100 nm PS physicochemical characteristics sufficiently to increase mucosal penetration and cellular uptake. However, in another study conjugating a 2 kDa homopolymer PEG to the surface of 100 and 200 nm PS nanoparticles, the diffusion coefficient of the particles through the human cervicovaginal mucus was increased by 20 and 381 times, respectively (Lai et al., 2007). Differences in the formulation design described by Lai et al. (2007) and a different mucosal model to our work may account for the disparity between the results. A 2 kDa PEG homopolymer has 2.3 times more PEG units than P80 which may explain the lower zeta potential for 100 nm 2 kDa PEG PS (−4 mV) compared to 100 nm P80-PS (−21.5 ± 1.7 mV). This results in a much denser (and thicker) PEG coating on the surface of the former particles than for the P80-PS. This is potentially relevant because it was shown by Wang et al. (2008b) that a low PEG molecular weight and a high PEG surface coverage were required for rapid penetration of mucus. Furthermore, the PEG in our study was adsorbed onto the PS surface rather than covalently attached and consequently may have been desorbed/displaced from the PS surface in the biological environment. Other variables may also affect the ability of PEG-modified nanoparticles to penetrate mucus and therefore reach epithelial cells; these include PEG molecular weight (longer PEG chains may increasingly interact with mucus fibres to reduce transmucosal movement of the nanoparticles (Wang et al., 2008b)), and nanoparticle core composition (which affects the particle surface charge) and/or effect of nanoparticle surface adsorbed proteins from the biological milieu (Goppert and Muller, 2005).

5.3.3. Lectin surface modification

Lectins are proteins or glycoproteins that can be purified from many plant sources such as tomatoes, jack bean and wheat germ. Lectins occur ubiquitously in nature and can recognise sugar residues on biological surfaces. Their selective affinity for biological surfaces may be useful for direct nose-to-brain drug delivery as described below.

In a recent study Wheat Germ Agglutinin (WGA) lectin was conjugated to coumarin-loaded PEG–poly(lactic acid) (PEG–PLA) nanoparticles (85–90 nm in diameter) and administered intranasally in rats (Gao et al., 2006). WGA binds to N-acetyl-D-glucosamine and sialic acid residues both of which are abundant on the nasal epithelial membrane (Ishikawa and Isayama, 1987). A 2-fold increase in coumarin was observed in the olfactory bulb, olfactory tract, cerebrum and cerebellum within 15 h of a single dose of lectin modified nanoparticles compared to unmodified nanoparticles, without any evidence of ciliotoxicity. The enhanced uptake was, however, thought to be poorly selective for coumarin delivery directly to the brain since similar increases were also observed in the blood. This was probably due to poor selectivity of WGA binding to the olfactory mucosa. In the later study from the same group using a slightly larger (90–100 nm) coumarin-loaded particles similar phenomena were observed (Gao et al., 2007a). In addition, the authors concluded that the olfactory and respiratory epithelia presented "different lectin binding profiles" which led to a "great affinity of WGA to the olfactory epithelium but a moderate degree to the respiratory epithelium." This was however, difficult to justify from the results presented due to the large variation in the control data. However, good evidence for the deep brain penetration of intact Vasoactive Intestinal Peptide (VIP; MW 3326) administered in PEG–PLA or WGA–PEG–PLA nanoparticle formulations was presented. VIP concentrations were found to increase in the olfactory bulb, cerebrum and cerebellum by 3.57-fold, 3.63-fold and 4.74-fold, respectively, when compared to *i.n.* solution; these values increased further to 5.66-fold, 6.61-fold and 7.74-fold for WGA–PEG–PLA nanoparticles. The increase in VIP concentrations also corresponded to improved memory function, as determined by the water maze behavioural test. This is the first evidence that has shown the ability of nanoparticles to protect a peptide drug from peptidase degradation in the nasal milieu, and furthermore, their enhanced pharmacological efficacy compared to control animals. It was not clear from the paper whether the nanoparticles and the WGA nanoparticles were transported intact to the CNS or whether the drug was released and then transported.

Considering site specific targeting to the olfactory region, some level of selectivity may be achieved from a delivery device, whereby the nanoparticle formulation is deposited directly on the olfactory epithelium; otherwise, drug absorption may occur through non-olfactory regions of the nose which could lead to different local or systemic effects. At the level of delivery system design, the currently predominantly used chitosan is a non-selective mucoadhesive molecule and there is consequently a need to find a ligand that would selectively interact with olfactory mucosa. Such approaches of an active drug targeting and inernalisation into the olfactory axons would promote deep brain penetration of the drug. To this end a recent study described the use of *Ulex europeus* agglutinin I (UEA I) which can recognise L-fucose residues on the apical surface of olfactory axons (Gao et al., 2007b). PEG-PLA nanoparticles (111 nm diameter) were conjugated to UEA I. Detection of coumarin (which was associated with the nanoparticles) in the different brain regions was 1.7 times increased after surface modification with the lectin compared to unmodified particles. In addition, fluorescence microscopy shows that the affinity was enhanced for UEA I-modified nanoparticles to the olfactory epithelium compared to the respiratory mucosa. Nevertheless, the visual evidence provided in the article showed a low magnification image of the nasal tissues. Higher magnification images could have enabled a detailed analysis of individual particles inside the olfactory epithelium, as well as details of the morphological condition of the olfactory epithelium. This would have provided yet stronger evidence for the uptake of individual particles into undamaged olfactory neurones.

5.3.4. Future strategies

Actively targeting the olfactory epithelium using lectin-conjugated nanoparticles may be problematic since lectins are in general toxic to mammalian cells (Fu et al., 1996; Reynoso-Camacho et al., 2003). Recent studies have shown that cholera toxin B subunit (CB) and synthetic 'homing peptides' could provide useful alternatives to lectins as direct nose-to-brain targeting ligands in the future.

For example, Zhang et al. (2008) nasally administered (15 μg/day) Nerve Growth Factor (NGF; MW 26.5 kDa) conjugated to CB (NGF-CB; MW 28.5 kDa) for 1 week in Alzheimer's disease modelled mice. The Morris Water Maze memory test showed that the escape latency times declined compared to β-amyloid treated control mice for NGF and CB-NGF. Moreover, significantly more ChAT positive neurones were found in the mice brains treated with NGF and CB-NGF compared to the β-amyloid controls. The authors speculated that the cholera toxin subunit of CB-NGF could bind to monosialoganglionside molecules on the olfactory axons and therefore enter the cell by endocytosis. The CB-NGF could then travel to the olfactory bulb by axoplasmic transportation to the target brain tissues. Hence, further proof of principle studies on CB conjugated to the exterior surface of nanoparticles may demonstrate similar targeting ability to deep brain tissues. CB is, however, immunogenic; as used in cholera vaccines (e.g. Dukoral®, SBL Vaccin AB, Sweden). The use of smaller synthetic 'homing peptides' may avoid this problem. Wan et al. (2009) have used a phage selection method to identify an 11 amino acid peptide (ACTTPHAWLCG) that enabled the phage to enter and translocate along olfactory axons 50 times more efficiently compared to a phage that dis-

played a random 11-mer peptide sequence after nasal application in the rat model. The translocation was competitively inhibited by addition of synthetic ACTTPHAWLCG peptide. This confirmed the selectivity of the ACTTPHAWLCG peptide for olfactory axon receptors. Hence, ACTTPHAWLCG and future phage selected direct nose-to-brain homing peptides could be conjugated to nanoparticles to improve direct nose-to-brain targeting.

Another strategy to increase direct nose-to-brain drug delivery has been to supplement the drug-containing formulation with other bioactive compounds. Dhuria et al. (2008) found that co-administration of 1% vasoconstrictor, phenylephrine, with HC-1 (MW 3562) or D-KTP (MW 337; an enzymatically stable D-analogue of the neuropeptide kyotorphin involved in pain regulation) increased the presence of these peptides significantly in the olfactory epithelium and in the olfactory bulbs compared to phenylephrine solutions. These increases in drug levels in the olfactory bulbs were associated with simultaneous decreases in absorption of drug into the blood. The authors concluded that addition of vasoconstrictors to the *i.n.* formulation can alter the distribution of the payload drug in favour of the olfactory route. They suggested that vasoconstriction of the phenylephrine reduced absorption of the HC-1 and D-KTP to the blood over the respiratory epithelium and therefore facilitated the transfer of drug through the olfactory system.

However, a similar study by Charlton et al. (2007b) did not concur as no changes were shown in drug distribution (of GR138950 an angiotensin antagonist; MW 611) in favour of the olfactory route in rats when ephedrine was added to the formulation. The difference may be arising from the fact that Dhuria et al., applied a short acting phenylephrine, which produces faster vasoconstriction in the nasal cavity whilst Charlton et al., used a longer acting sympathomimetic vasoconstrictor, ephedrine, which also has a longer onset of action. Consequently the ability of ephedrine to reduce systemic drug absorption via vasoconstriction may have been reduced compared to phenylephrine.

6. Conclusions

In summary an analysis of the available literature has revealed that smaller water soluble drugs (<1000 Da) may have rapid access to CSF compared to lipophilic molecules, since the former are less likely to be transported via the neuronal and supporting cells of the olfactory epithelium after nasal application. Nanoparticles may be taken into the neurones and supporting cells by a number of endocytic mechanisms. However, nanoparticles larger than 100 nm are thought to have a restricted access to the brain via the intra-axonal route because their diameter increasingly exceeds that of the axons in the *filia olfactoria*.

There is evidence to suggest that direct nose-to-brain transport using synthetic nanoparticles is possible, may be, even to therapeutic levels in animal models and in humans. Nevertheless, it is still unclear whether: (i) whole particles are selectively taken into the olfactory epithelial cells and transported; or (ii) nanoparticles release the incorporated drug in vicinity of the apical surface of the olfactory cells. Clear evidence of uptake of synthetic nanoparticles into olfactory neurones beyond the basement membrane has been shown by De Lorenzo (1960).

Furthermore, it has been shown that nanoparticles may cause cellular and subcellular damage to biological systems. The challenge now is to improve the transfer efficiency of drug loaded particles from the olfactory epithelium to the brain in order to safely, predictably and successfully reach therapeutically relevant levels in the target brain regions of humans.

It can be concluded that more fundamental studies are required. Future studies could use appropriately labelled nanoparticles composed of non-biodegradable polymer which could determine the route of these particles from the olfactory epithelium to the brain and possibly their further fate through clearance. Other information, such as the transport rate of different sized nanoparticles to the brain and an evaluation of the effect of different surface modifications and introduction of specific ligands would provide useful information and progress in the field.

References

Adams, G.L., Boies, L.R., Hilger, P.A., 1989. Boies's Fundamentals of Otolaryngology a Textbook of Ear, Nose and Throat Diseases. Saunders, Philadelphia, pp. 177–195.

Almeida, A.J., Alpar, H.O., Brown, M.R.W., 1993. Immune response to nasal delivery of antigenically intact tetanus toxoid associated with poly(L-lactic acid) microspheres in rats, rabbits and guinea pigs. J. Pharm. Pharmacol. 45, 198–203.

Baier, P.C., Weinhold, S.L., Huth, V., Gottwald, B., Ferstl, R., Hinze-Selch, D., 2008. Olfactory dysfunction in patients with narcolepsy with cataplexy is restored by intranasal orexin a (hypocretin-1). Brain 131, 2734–2741.

Behrens, I., Vila Pena, A.I., Alonso, M.J., Kissel, T., 2002. Comparative uptake studies of bioadhesive nanoparticles in human intestinal cell lines and rats: the effect of mucus on particle adsorption and transport. Pharm. Res. 19, 1185–1193.

Benedict, C., Kern, W., Schultes, B., Born, J., Hallschmid, M., 2008. Differential sensitivity of men and women to anorexigenic and memory-improving effects of intranasal insulin. J. Clin. Endocrinol. Metab. 93, 1339–1344.

Betbeder, D., Sperandio, S., Latapie, J.P., de Nadai, J., Etienne, A., Zajac, J.M., Frances, B., 2000. Biovector (tm) nanoparticles improve antinociceptive efficacy of nasal morphine. Pharm. Res. 17, 743–748.

Bodian, D., Howe, H.A., 1941. The rate of progression of poliomyelitis virus in nerves. Bull. Johns Hopkins Hosp. 69, 79–85.

Born, J., Lange, T., Kern, W., McGregor, G.P., Bickel, U., Fehm, H.L., 2002. Sniffing neuropeptide: a transnasal approach to the human brain. Nat. Neurosci. 5, 514–516.

Brand, G., 2006. Olfactory/trigeminal interactions in nasal chemoreception. Neurosci. Biobehav. Rev. 30, 908–917.

Brodbelt, A., Stoodley, M., 2007. CSF pathways: a review. Br. J. Neurosurg. 21, 510–520.

Brooking, J., Davis, S.S., Illum, L., 2001. Transport of nanoparticles across the rat nasal mucosa. J. Drug Target. 9, 267–279.

Burd, G.D., 1991. Development of the olfactory nerve in the African clawed frog, *Xenopus laevis*. 1. Normal development. J. Comp. Neurol. 304, 123–134.

Charlton, S., Jones, N.S., Davis, S.S., Illum, L., 2007a. Distribution and clearance of bioadhesive formulations from the olfactory region in man: effect of polymer type and nasal delivery device. Eur. J. Pharm. Sci. 30, 295–302.

Charlton, S., Davis, S.S., Illum, L., 2007b. Evaluation of effect of ephedrine on the transport of drugs from the nasal cavity to the systemic circulation and the central nervous system. J. Drug Target. 15, 370–377.

Chen, F., Zhang, Z.-R., Yuan, F., Qin, X., Wang, M., Huang, Y., 2008. In vitro and in vivo study of N-trimethyl chitosan nanoparticles for oral protein delivery. Int. J. Pharm. 349, 226–233.

Chou, K.-J., Donovan, M.D., 1997. Distribution of antihistamines into the CSF following intranasal delivery. Biopharm. Drug Dispos. 18, 335–346.

Conner, S.D., Schmid, S.L., 2003. Regulated portals of entry into the cell. Nature 422, 37–44.

Costantino, H.R., Illum, L., Brandt, G., Johnson, P., Quay, S.C., 2007. Intranasal delivery: physiochemical and therapeutic aspects. Int. J. Pharm. 337, 1–24.

Dahlin, M., Jansson, B., Bjork, E., 2001. Levels of dopamine in blood and brain following nasal administration to rats. Eur. J. Pharm. Sci. 14, 75–80.

Dalpiaz, A., Gavini, E., Colombo, G., Russo, P., Bortolotti, F., Ferraro, L., Tanganelli, S., Scatturin, A., Menegatti, E., Giunchedi, P., 2008. Brain uptake of an anti-ischemic agent by nasal administration of microparticles. J. Pharm. Sci. 97, 4889–4903.

De Lorenzo, A., 1960. Electron microscopy of the olfactory and gustatory pathways. Ann. Otol. Rhinol. Laryngol. 68, 410–420.

De Lorenzo, A., 1970. Taste and Smell in Vertebrates. J&A Churchill, London, pp. 151–176.

Derad, I., Willeke, K., Pietrowsky, R., Born, J., Fehm, H.L., 1998. Intranasal angiotensin iI directly influences central nervous regulation of blood pressure. Am. J. hypertens. 11, 971–977.

Des Rieux, A., Ragnarsson, E.G.E., Gullberg, E., Preat, V., Schneider, Y.-J., Artursson, P., 2005. Transport of nanoparticles across an in vitro model of the human follicle associated epithelium. Eur. J. Pharm. Sci. 25, 455–465.

Dhuria, S., Hanson, L., Frey, W.I., 2008. Intranasal drug targeting of hypocretin-1 (orexin-a) to the central nervous system. J. Pharm. Sci. (Published Online).

Edeling, M.A., Smith, C., Owen, D., 2006. Life of a clathrin coat: insights from clathrin and AP structures. Nat. Rev. Mol. Cell. Biol. 7, 32–44.

Elder, A., Gelein, R., Silva, V., Feikert, T., Opanashuk, L., Carter, J., Potter, R., Maynard, A., Finkelstein, J., Oberdorster, G., 2006. Translocation of inhaled ultrafine manganese oxide particles to the central nervous system. Environ. Health Perspect. 114, 1172–1178.

Fu, T., Burbage, C., Tagge, E.P., Brothers, T., Willingham, M.C., Frankel, A.E., 1996. Ricin toxin contains three lectin sites which contribute to its in vivo toxicity. Int. J. Immunopharmacol. 18, 685–692.

Gao, X., Wu, B., Zhang, Q., Chen, J., Zhu, J., Zhang, W., Rong, Z., Chen, H., Jiang, X., 2007a. Brain delivery of vasoactive intestinal peptide enhanced with the nanoparticles conjugated with wheat germ agglutinin following intranasal administration. J. Control. Release 121, 156–167.

Gao, X., Chen, J., Tao, W., Zhu, J., Zhang, Q., Chen, H., Jiang, X., 2007b. Uea I-bearing nanoparticles for brain delivery following intranasal administration. Int. J. Pharm. 340, 207–215.
Gao, X.L., Tao, W.X., Lu, W., Zhang, Q.Z., Zhang, Y., Jiang, X.G., Fu, S.K., 2006. Lectin-conjugated PEG–PLA nanoparticles: preparation and brain delivery after intranasal administration. Biomaterials 27, 3482–3490.
Gartner, L.P., Hiatt, J.L., 2000. Color Atlas of Histology. Lippincott Williams & Wilkins, Philadelphia.
Goppert, T.M., Muller, R.H., 2005. Polysorbate-stabilized solid lipid nanoparticles as colloidal carriers for intravenous targeting of drugs to the brain: comparison of plasma protein adsorption patterns. J. Drug Target. 13, 179–187.
Gumbiner, B., 1987. Structure, biochemistry, and assembly of epithelial tight junctions. Am. J. Physiol. 253, C749–C758.
Harush-Frenkel, O., Rozentur, E., Benita, S., Altschuler, Y., 2008. Surface charge of nanoparticles determines their endocytic and transcytotic pathway in polarized MDCK cells. Biomacromolecules 9, 435–443.
Huang, Y., Donovan, M.D., 1996. Microsphere transport pathways in the rabbit nasal mucosa. Int. J. Pharm. Adv. 1, 298–309.
Illum, L., 2000. Transport of drugs from the nasal cavity to the central nervous system. Eur. J. Pharm. Sci. 11, 1–18.
Illum, L., 2004. Is nose-to-brain transport of drugs in man a reality? J. Pharm. Pharmacol. 56, 3–17.
Illum, L., 2006. Nanoparticle systems for nasal delivery of drugs: a real improvement over simple systems? J. Pharm. Sci. 96, 473–483.
Ishikawa, H., Isayama, Y., 1987. Evidence for sialyl glycoconjugates as receptors for *Bordetella bronchiseptica* on swine nasal mucosa. Infect. Immun. 55, 1607–1609.
Jogani, V.V., Shah, P.J., Mishra, P., Mishra, A.K., Misra, A.R., 2008. Intranasal mucoadhesive microemulsion of tacrine to improve brain targeting. Alzheimer Dis. Assoc. Disord. 22, 116–124.
Jones, A.T., 2008. Gateways and tools for drug delivery: endocytic pathways and the cellular dynamics of cell penetrating peptides. Int. J. Pharm. 354, 34–38.
Kaliner, M., Marom, Z., Patow, C., Shelhamer, J., 1984. Human respiratory mucus. J. Allergy Clin. Immunol. 73, 318–323.
Kern, W., Born, J., Schreiber, H., Fehm, H.L., 1999. Central nervous system effects of intranasally administered insulin during euglycemia in men. Diabetes 48, 557–563.
Kumar, M., Misra, A., Babbar, A.K., Mishra, A.K., Mishra, P., Pathak, K., 2008a. Intranasal nanoemulsion based brain targeting drug delivery system of risperidone. Int. J. Pharm. 358, 285–291.
Kumar, M., Misra, A., Mishra, A.K., Mishra, P., Pathak, K., 2008b. Mucoadhesive nanoemulsion-based intranasal drug delivery system of olanzapine for brain targeting. J. Drug Target. 16, 806–814.
Lai, S.K., O'Hanlon, D.E., Harrold, S., Man, S.T., Wang, Y.Y., Cone, R., Hanes, J., 2007. Rapid transport of large polymeric nanoparticles in fresh undiluted human mucus. Proc. Natl. Acad. Sci. U.S.A. 104, 1482–1487.
Lenaerts, V., Gurny, R., 1990. Bioadhesive Drug Delivery Systems. CRC Press, Boca Raton, USA, pp. 12–13.
Lledo, P.M., Gheusi, G., Vincent, J.D., 2005. Information processing in the mammalian olfactory system. Physiol. Rev. 85, 281–317.
Ma, Z., Lim, L.-Y., 2003. Uptake of chitosan and associated insulin in Caco-2 cell monolayers: a comparison between chitosan molecules and chitosan nanoparticles. Pharm. Res. 20, 1812–1819.
Matsuzaki, O., 1995. Numbers of olfactory receptor-cells and pine structure of olfactory nerves in various birds. Zool. Sci. 12, 117–123.
Mayor, S., Pagano, R.E., 2007. Pathways of clathrin-independent endocytosis. Nat. Rev. Mol. Cell. Biol. 8, 603–612.
McMartin, C., Hutchinson, L.E.F., Hyde, R., Peters, G.E., 1987. Analysis of structural requirements for the absorption of drugs and macromolecules from the nasal cavity. J. Pharm. Sci. 76, 535–540.
Menache, M.G., Hanna, L.M., Gross, E.A., Lou, S.R., Zinreich, S.J., Leopold, D.A., Jarabek, A.M., Miller, F.J., 1997. Upper respiratory tract surface areas and volumes of laboratory animals and humans: considerations for dosimetry models. J. Toxicol. Environ. Health 50, 475–506.
Merkus, F.W.H.M., van den Berg, M.P., 2007. Can nasal drug delivery bypass the blood–brain barrier? Questioning the direct transport theory. Drugs. R. D. 8, 133–144.
Merkus, P., Guchelaar, H.-J., Bosch, A., Merkus, F.W.H.M., 2003. Direct access of drugs to the human brain after intranasal drug administration? Neurology 60, 1669–1671.
Miaczynska, M., Stenmark, H., 2008. Mechanisms and functions of endocytosis. J. Cell. Biol. 180, 7–11.
Mistry, A., Glud, S.Z., Kjems, J., Randel, J., Howard, K.A., Stolnik, S., Illum, L., 2009. The effect of physicochemical properties on intranasal nanoparticle transit into murine olfactory epithelium. J. Drug Target, doi:10.1080/1061186093055470.
Mistry A., 2008. The development and application of biological models for evaluation of direct nose-to-brain drug delivery systems. Thesis, University of Notttingham, UK.
Miyamoto, M., Natsume, H., Satoh, I., Ohtake, K., Yamaguchi, M., Kobayashi, D., Sugibayashi, K., Morimoto, Y., 2001. Effect of poly-L-arginine on the nasal absorption of FITC-dextran of different molecular weights and recombinant human granulocyte colony-stimulating factor (RHG-CSF) in rats. Int. J. Pharm. 226, 127–138.
Moran, D.T., Rowley, J.C., Jafek, B.W., Lovell, M.A., 1982. The fine-structure of the olfactory mucosa in man. J. Neurocytol. 11, 721–746.
Morrison, E.E., Costanzo, R.M., 1992. Morphology of olfactory epithelium in humans and other vertebrates. Microsc. Res. Tech. 23, 49–61.
Mygind, N., 1979. Nasal Allergy. Blackwell Scientific, Oxford.
Oberdörster, G., Sharp, Z., Atudorei, V., Elder, A., Gelein, R., Kreyling, W., Cox, C., 2004. Translocation of inhaled ultrafine particles to the brain. Inhal. Toxicol. 16, 437–445.
Oberdörster, G., Oberdörster, E., Oberdörster, J., 2005. Nanotoxicology: an emerging discipline evolving from studies of ultrafine particles. Environ. Health Perspect. 113, 823–839.
Pardridge, W.M., 1999. Non-invasive drug delivery to the human brain using endogenous blood–brain barrier transport systems. Pharm. Sci. Technol. Today 2, 49–59.
Pietrowsky, R., Struben, C., Molle, M., Fehm, H.L., Born, J., 1996a. Brain potential changes after intranasal vs. intravenous administration of vasopressin: evidence for a direct nose brain pathway for peptide effects in humans. Biol. Psychiatry 39, 332–340.
Pietrowsky, R., Thiemann, A., Kern, W., Fehm, H.L., Born, J., 1996b. A nose–brain pathway for psychotropic peptides: evidence from a brain evoked potential study with cholecystokinin. Psychoneuroendocrinology 21, 559–572.
Proetz, A., 1953. Applied Physiology of the Nose. Annals Publishing Company, St. Louis.
Rejman, J., Oberle, V., Zuhorn, I.S., Hoekstra, D., 2004. Size-dependent internalization of particles via the pathways of clathrin- and caveolae-mediated endocytosis. Biochem. J. 377, 159–169.
Reynoso-Camacho, R., Gonzalez De Mejia, E., Loarca-Pina, G., 2003. Purification and acute toxicity of a lectin extracted from tepary bean (*Phaseolus acutifolius*). Food Chem. Toxicol. 41, 21–27.
Rhodin, J.A.G., 1974. Histology a Text and Atlas. Oxford University Press, New York, p. 611.
Rothen-Rutishauser, B.M., Schurch, S., Haenni, B., Kapp, N., Gehr, P., 2006. Interaction of fine particles and nanoparticles with red blood cells visualized with advanced microscopic techniques. Environ. Sci. Technol. 40, 4353–4359.
Sadeghi, A.M.M., Dorkoosh, F.A., Avadi, M.R., Weinhold, M., Bayat, A., Delie, F., Gurny, R., Larijani, B., Rafiee-Tehrani, M., Junginger, H.E., 2008. Permeation enhancer effect of chitosan and chitosan derivatives: comparison of formulations as soluble polymers and nanoparticulate systems on insulin absorption in Caco-2 cells. Eur. J. Pharm. Biopharm. 70, 270–278.
Sakane, T., Akizuki, M., Taki, Y., Yamashita, S., Sezaki, H., Nadai, T., 1995. Direct drug transport from the rat nasal cavity to the cerebrospinal fluid: the relation to the molecular weight of drugs. J. Pharm. Pharmacol. 47, 379–381.
Sakane, T., Akizuki, M., Yamashita, S., Nadai, T., Hashida, M., Sezaki, H., 1991. The transport of a drug to the cerebrospinal fluid directly from the nasal cavity the relation to the lipophilicity of the drug. Chem. Pharm. Bull. 39, 2456–2458.
Sakane, T., Akizuki, M., Yamashita, S., Sezaki, H., Nadai, T., 1994. Direct drug transport from the rat nasal cavity to the cerebrospinal fluid: the relation to the dissociation of the drug. J. Pharm. Pharmacol. 46, 378–379.
Semmler, M., Seitz, J., Erbe, F., Mayer, P., Heyder, J., Oberdorster, G., Kreyling, W.G., 2004. Long-term clearance kinetics of inhaled ultrafine insoluble iridium particles from the rat lung, including transient translocation into secondary organs. Inhal. Toxicol. 16, 453–459.
Shipley, M.T., 1985. Transport of molecules from nose to brain: transneural anterograde and retrograde labelling in the rat olfactory system by wheat germ agglutinin–horseradish peroxidase applied to the nasal epithelium. Brain Res. Bull. 15, 129–142.
Smith, J., Wood, E., Dornish, M., 2004. Effect of chitosan on epithelial cell tight junctions. Pharm. Res. 21, 43–49.
Smith, J.M., Dornish, M., Wood, E.J., 2005. Involvement of protein kinase c in chitosan glutamate-mediated tight junction disruption. Biomaterials 26, 3269–3276.
Sorkin, A., 2004. Cargo recognition during clathrin-mediated endocytosis: a team effort. Curr. Opin. Cell Biol. 16, 392–399.
Thorne, R.G., Pronk, G.J., Padmanabhan, V., Frey, W.H., 2004. Delivery of insulin-like growth factor-i to the rat brain and spinal cord along olfactory and trigeminal pathways following intranasal administration. Neuroscience 127, 481–496.
Tucker, D., 1971. Nonolfactory Responses from the Nasal Cavity: Jacobson's Organ and the Trigeminal System. Springer, Heidelberg, pp. 151–181.
Van Itallie, C.M., Anderson, J.M., 2006. Claudins and epithelial paracellular transport. Annu. Rev. Physiol. 68, 403–429.
Wan, X.M., Chen, Y.P., Xu, W.R., Yang, W.J., Wen, L.P., 2009. Identification of nose-to-brain homing peptide through phage display. Peptides 30, 343–350.
Wang, B., Feng, W.Y., Wang, M., Shi, J.W., Zhang, F., Ouyang, H., Zhao, Y.L., Chai, Z.F., Huang, Y.Y., Xie, Y.N., Wang, H.F., Wang, J., 2007. Transport of intranasally instilled fine Fe_2O_3 particles into the brain: micro-distribution, chemical states, and histopathological observation. Biol. Trace Elem. Res. 118, 233–243.
Wang, J., Liu, Y., Jiao, F., Lao, F., Li, W., Gu, Y., Li, Y., Ge, C., Zhou, G., Li, B., Zhao, Y., Chai, Z., Chen, C., 2008a. Time-dependent translocation and potential impairment on central nervous system by intranasally instilled TiO(2) nanoparticles. Toxicology 254, 82–90.
Wang, X., He, H., Leng, W., Tang, X., 2006. Evaluation of brain-targeting for the nasal delivery of estradiol by the microdialysis method. Int. J. Pharm. 317, 40–46.
Wang, Y.-Y., Lai, S.K., Suk, J.S., Pace, A., Cone, R., Hanes, J., 2008b. Addressing the PEG mucoadhesivity paradox to engineer nanoparticles that "Slip". Through the human mucus barrier. Angew. Chem. Int. Ed. Engl. 47, 9726–9729.
Westin, U., Piras, E., Jansson, B., Bergstrom, U., Dahlin, M., Brittebo, E., Bjork, E., 2005. Transfer of morphine along the olfactory pathway to the central nervous system after nasal administration to rodents. Eur. J. Pharm. Sci. 24, 565–573.

Xia, T., Kovochich, M., Liong, M., Zink, J.I., Nel, A.E., 2008. Cationic polystyrene nanosphere toxicity depends on cell-specific endocytic and mitochondrial injury pathways. ACS Nano 2, 85–96.
Xiang, S.D., Scholzen, A., Minigo, G., David, C., Apostolopoulos, V., Mottram, P.L., Plebanski, M., 2006. Pathogen recognition and development of particulate vaccines: does size matter? Methods 40, 1–9.
Zhang, Q., Jiang, X., Jiang, W., Lu, W., Su, L., Shi, Z., 2004. Preparation of nimodipine-loaded microemulsion for intranasal delivery and evaluation on the targeting efficiency to the brain. Int. J. Pharm. 275, 85–96.
Zhang, Q., Liu, Y., Yang, N., Wan, X., Zuo, P., 2008. Nasal administration of cholera toxin B subunit-nerve growth factor improves the space learning and memory abilities in beta-amyloid protein (25–35)-induced amnesic mice. Neuroscience 155, 234–240.
Zhang, Q.Z., Zha, L.S., Zhang, Y., Jiang, W.M., Lu, W., Shi, Z.Q., Jiang, X.G., Fu, S.K., 2006. The brain targeting efficiency following nasally applied MPEG–PLA nanoparticles in rats. J. Drug Target. 14, 281–290.