Direct nose to brain drug delivery via integrated nerve pathways bypassing the blood–brain barrier: an excellent platform for brain targeting

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Introduction: The blood–brain barrier (BBB) represents a stringent barrier for delivery of neurotherapeutics in vivo. An attempt to overcome this barrier is represented by the direct transport of drugs from the nose to the brain along the olfactory and trigeminal nerve pathways. These nerve pathways initiate in the nasal cavity at olfactory neuroepithelium and terminate in the brain. An enormous range of neurotherapeutics, both macromolecules and low molecular weight drugs, can be delivered to the central nervous system (CNS) via this route. Areas covered: Present review highlights the literature on the anatomy-physiology of the nasal cavity, pathways and mechanisms of neurotherapeutic transport across nasal epithelium and their biofate and various strategies to enhance direct nose to brain drug delivery. The authors also emphasize a variety of drug molecules and carrier systems delivered via this route for treating CNS disorders. Patents related to direct nose to brain drug delivery systems have also been listed.

Expert opinion: Direct nose to brain drug delivery system is a practical, safe, non-invasive and convenient form of formulation strategy and could be viewed as an excellent alternative approach to conventional dosage forms. Existence of a direct transport route from the nasal cavity to the brain, bypassing the BBB, would offer an exciting mode of delivering neurotherapeutic agents.

Keywords: blood–brain barrier, mucociliary clearance, nasal drug delivery, nose to brain, olfactory transport


1. Introduction

Traditionally, the nasal route has been exploited for delivery of drugs for the treatment of local diseases like nasal allergy, sinusitis, nasal infections and nasal congestion. In the last few decades, the nasal route has attracted wide attention as a reliable, safe, non-invasive and convenient route to accomplish faster and higher levels of drug absorption [1]. The crucial reasons behind the interest in the nasal route are the highly vascularized epithelium of nasal mucosa, porous endothelial membrane, its ready accessibility, its large surface area for rapid drug absorption, rapid onset of action, lower enzyme levels compared with gastrointestinal tract and liver, high total blood flow per cm² and direct drug transport to systemic circulation and to the brain, thereby avoiding first-pass hepatic metabolism and enhancing bioavailability [2].

Conventional drug delivery methods fall short in delivering a number of therapeutic agents to the brain efficiently. The blood–brain barrier (BBB) and
2. Nasal structure and physiology

2.1 Nasal cavity

Structurally, the nose is divided into two nasal cavities via a midline septum. The nasal cavity is about 12 cm long; the volume of each nasal cavity is 13 ml and has a surface area of around 150 cm² [17]. Each cavity consists of three different regions, namely the vestibule, the olfactory region, and the respiratory region. The nasal vestibule consists of the region just inside the nostrils with an area of about 0.6 cm². The respiratory region contains three nasal turbinates, the superior, the middle, and the inferior turbinate. These turbinates project from the lateral wall of each half of the nasal cavity. These turbinates produce turbulent airflow through the nasal passages which ensure a better contact between inhaled air and mucosal surface. The olfactory region is situated at the roof of the nasal cavity in humans and covers about 10% of the total surface area of the nasal cavity.

Functionally, the nasal cavity plays an important protective role to filter, warm and humidify the inhaled air before it reaches the lower airways. It provides a supply and conditioning of air to lungs. Any inhaled particles or microorganisms are trapped by the hair of the nasal vestibule or by the mucus layer covering the respiratory area of the nasal cavity. The mucociliary clearance mechanism of the mucus layer gradually carries such particulates to the back of the throat, down the esophagus, and further into the gastrointestinal tract. Nasal mucosa also have the metabolic capability of converting endogenous materials into compounds that are eliminated more readily [18,19].

blood–cerebrospinal fluid (BCB) barrier restrict the transport of drugs from systemic circulation into the central nervous system (CNS). Although the BBB serves to protect the brain and spinal cord from a variety of pathogens and toxicants, it also presents a significant barrier to many of the xenobiotics in the treatment of CNS disorders. Hence, many therapeutic agents may have been abandoned because of their inability to achieve sufficient levels in brain through systemic circulation. Inevitably, a number of invasive strategies like intraparenchymal injections to bypass the blood–brain barrier (BBB) and rapidly target neurotherapeutics directly to the central nervous system (CNS), as it offers a unique connection between nasal cavity and brain, bypassing the BBB.

Direct nose to brain drug delivery has been proved to be an excellent platform for brain targeting through surface engineering of neurotherapeutic-loaded carrier systems resulting in enhanced product performance.

Scientists from various disciplines have been harnessed around the world for the exploitation of their potential applications in the core areas of pharmaceutical sciences.

This box summarizes key points contained in the article.
to the nasopharynx. Therefore, particles applied on the nasal respiratory mucosa will be transported on the mucus to the back of throat. The mucus flow rate is 5 mm/min (with a range of 0.5 – 23.6 mm/min) and hence the mucus layer is renewed every 15 – 20 min. In humans, mucociliary flow can be measured by means of gamma scintigraphy or the saccharine clearance test [17-19].

2.3 Olfactory epithelium and neuronal supply to the nasal cavity
The epithelial layer of the olfactory region consists of three types of cells, namely the olfactory neural cells, the sustentacular or supporting cells and the basal cells. Basal cells are progenitor cells of sustentacular or supporting cells, which also provide mechanical support through anchorage to other cells. The olfactory neural cells, or axons, are unmyelinated cells and are interspaced between supporting cells. They originate at the olfactory bulb and terminate at the apical surface of the olfactory neuroepithelium (Figure 3) [17]. The average diameter of olfactory axons in 2-month-old rabbits, as studied by electron microscopy, is ~ 200 nm, however, many of the axons have diameters of < 100 nm. Thus, 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 nose to brain drug transport route via the olfactory epithelium [17,20].

The olfactory knob (or vesicle) projects out from and above the apical surface of olfactory epithelium. Approximately 10 – 23 cilia project from the basal bodies of the knob, each of length up to 200 µm. The cilia are non-motile in the olfactory region (in contrast to respiratory tissue) as they lack the dynein arms which contain Mg2+-ATPase, required for generation of force for ciliary motility [17].

The lamina propria of olfactory epithelium, which is situated underneath the epithelial layer(s), contains blood supply, mucus secreting acinar glands (Bowman’s glands), nasal lymphatics and neuronal supply that consists of olfactory axon bundles, autonomic nerve fibers and the maxillary branch of trigeminal nerve. Bowman’s glands are under the control of the parasympathetic nervous system. These acinar glands produce nasal secretions in the lamina propria and secrete them through a narrow tube-like opening into the luminal space [17].

3. Pathways and mechanisms
While the precise mechanisms underlying intranasal drug delivery to the CNS are not entirely understood yet, an accumulating body of evidences demonstrates that pathways involving nerves connecting the nasal passages to the brain and spinal cord are important. Additionally, pathways involving the vasculature, CSF and lymphatic system have been employed in transport of molecules from nasal cavity to the CNS. It is possible that a combination of these pathways is
responsible, although one pathway may predominate, depending on the properties of neurotherapeutics, the characteristics of formulations and the delivery device used.

3.1 Olfactory nerve pathway

In order for a drug to travel from the olfactory region in the nasal cavity to the CSF or brain parenchyma, it has to traverse the nasal olfactory epithelium and, depending on the pathway followed, also the arachnoid membrane surrounding the subarachnoid space. In principle, one can envisage three different pathways across the olfactory epithelium: i) transcellular pathway; especially across the sustentacular cells, most likely by receptor-mediated endocytosis, fluid phase endocytosis or by passive diffusion. Passive diffusion is most likely for more lipophilic drugs. It is mediated rapidly and at a high rate. This route is responsible for the transport of lipophilic drugs and it shows rate dependency on their lipophilicity, ii) paracellular pathway; through tight junctions between sustentacular cells or the so-called clefts between sustentacular cells and olfactory neurons. Nasal absorption of hydrophilic drugs most probably occurs by diffusion through aqueous channels (pores). This pathway is slow and passive. This route is responsible for transport of hydrophilic drugs and it shows rate dependency on the molecular weight of a drug. Drugs with a molecular weight up to 1000 Da without absorption enhancer shows good bioavailability which can be extended to drugs with molecular weight up to 6000 Da with absorption enhancer, and iii) the olfactory nerve pathway; where drug is taken up into the neuronal cell by endocytosis or pinocytosis mechanisms and transported by intracellular axonal transport to the olfactory bulb [21,22]. Thus, the different modes of drug transport across the nasal olfactory epithelium are: transcellular passive diffusion (Figure 4A), paracellular passive diffusion (Figure 4B), carrier-mediated transport (Figure 4C), transcytosis (Figure 4D) and efflux transport (Figure 4E) [23].

3.2 Trigeminal nerve pathway

An important pathway connecting nasal passages to the CNS involves the trigeminal nerve (the largest nerve among all cranial nerves), which innervates the respiratory and olfactory epithelium of nasal passages and enters the CNS in the pons (Figure 5). A small portion of trigeminal nerve also terminates in the olfactory bulbs. The trigeminal nerve communicates sensory information from the nasal cavity, oral cavity, eyelids and cornea to the CNS via the ophthalmic division (V1), the maxillary division (V2) or the mandibular division (V3) of

Table 1. Advantages and limitations of nose to brain drug delivery system.

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Limitations</th>
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<tbody>
<tr>
<td>It is a rapid, safe, non-invasive and convenient method of drug delivery</td>
<td>Rapid elimination of drug substances from nasal cavity due to mucociliary clearance</td>
</tr>
<tr>
<td>It avoids drug degradation in gastrointestinal tract, particularly peptide drugs</td>
<td>Absorption enhancers used in formulation may create mucosal toxicity</td>
</tr>
<tr>
<td>It avoids hepatic first-pass metabolism and gut-wall metabolism of drugs, allowing enhanced bioavailability</td>
<td>There is a great deal of variability in the concentration attainable in different regions of brain and spinal cord</td>
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<tr>
<td>It bypasses BBB, thereby providing CNS targeted drug delivery, thereby reducing systemic exposure of drugs and associated systemic side effects</td>
<td>High molecular weight of drugs may result in decreased permeability across nasal mucosa</td>
</tr>
<tr>
<td>Therapeutic agent does not require any modification for being delivered via nose to brain drug delivery platform</td>
<td>Some therapeutic agents may cause irritation to nasal mucosa or may be susceptible to enzymatic degradation and metabolism in the nasal milieu at mucosal surface</td>
</tr>
<tr>
<td>Rapid drug absorption and quick onset of action can be achieved through highly vascularized and permeable structure of nasal mucosa</td>
<td>Nasal congestion due to cold or allergic condition may interfere with this technique of drug delivery</td>
</tr>
<tr>
<td>Better patient compliance, because self-medication is also possible with this route of drug administration</td>
<td>Frequent use of this route may cause mucosal damage like infection or anosmia</td>
</tr>
<tr>
<td>It serves as an alternative route for parenteral administration, especially for protein and peptide drugs or even for stem cells</td>
<td>Mechanical loss of the dosage form could occur due to improper technique of administration</td>
</tr>
<tr>
<td>Shows excellent bioavailability for low molecular weight drugs</td>
<td>Mechanisms of drug transport are still unclear</td>
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Figure 2. Ciliary motion and mucus layer that allows mucociliary clearance. A) Effective stroke, B) recovery stroke, C) gel layer, D) sol layer, E) direction of gel layer. Adapted from [17] with permission of copyright holder, Elsevier, Amsterdam.

Table 1. Advantages and limitations of nose to brain drug delivery system.
trigeminal nerve. The former two have only sensory function while later have both sensory as well as motor function. The ophthalmic and maxillary branches of trigeminal nerve are important for nose to brain drug delivery as neurons from these branches pass directly through the nasal mucosa [24].

A unique feature of the trigeminal nerve is that it enters the brain from the respiratory epithelium of the nasal passages at two sites: i) through anterior lacerated foramen near the pons and ii) through the cribriform plate near olfactory bulb, creating entry points into both caudal and rostral brain areas following intranasal administration. While there are no published reports on the ensheathing cells and channels associated with trigeminal nerve comparable with that observed with the olfactory nerves, these anatomical features may be present along the trigeminal nerve. It is also possible that the other nerves that innervate the face and head, such as facial nerve, or other sensory structures in the nasal cavity, such as the Grueneberg ganglion, may provide entry points for intranasally administered neurotherapeutics into the CNS [24].

Thorne et al. [25] demonstrated, for the first time, CNS delivery of insulin-like growth factor-I (125I-IGF-I), a protein neurotropic factor, following intranasal administration and observed the high levels of radioactivity in trigeminal nerve branches, trigeminal ganglion, pons and olfactory bulbs, consistent with delivery along both olfactory and trigeminal nerves. Since one portion of the trigeminal neural pathway enters the brain through the cribriform plate along the olfactory pathway, it is difficult to differentiate whether intranasally administered drugs reach the olfactory bulbs and other rostral brain areas (anterior olfactory nucleus and frontal cortex) via the olfactory pathway, or brainstem and spinal cord regions via trigeminal pathway, or if both extracellular pathways are involved, bypassing the BBB.

3.3 Pathways involving cerebrospinal fluid and nasal lymphatics

Pathways connecting the subarachnoid space containing CSF, perineurial spaces encompassing olfactory nerves and nasal lymphatics provide a gateway for intranasally applied therapeutics to the CSF and other areas of the CNS [24]. Studies
document that radiolabeled tracers injected into the CSF in cerebral ventricles or subarachnoid space drain to the underside of olfactory bulbs, into channels associated with olfactory nerves traversing the cribriform plate, and reach the nasal lymphatic system and cervical lymph nodes [26]. Walter et al. [27] investigated the direct connections between CSF and nasal lymphatics as demonstrated from their experiment on rat model. They injected antigen into the subarachnoid space and succeeded in obtaining the evidence that this antigen also appears in both superficial and deep cervical lymph nodes, suggesting the drainage of this antigen from the subarachnoid space to extracranial lymphatic vessels along olfactory nerves.

These pathways also ensure the access of drugs to the CNS after intranasal administration, moving from nasal passages to the CSF, the brain interstitial spaces and perivascular spaces for distribution throughout the brain. Pathways between nasal passages and the CSF are still important and functional in humans as evidenced by the report that neurotherapeutics are directly delivered to the CSF after intranasal delivery, without entering the blood to a significant extent [28].

Many intranasally applied neurotherapeutics rapidly enter the CSF, and this transport is dependent on the lipophilicity, molecular weight and degree of ionization of these therapeutic molecules [28,29]. Evaluating distribution into the CSF can provide information on mechanism of nose to brain drug delivery. For example, observing a decreased concentration gradient from CSF to brain tissues or observing distribution of therapeutics to the brain areas distant from the olfactory bulbs are consistent with distribution via CSF [30]. However, the trigeminal nerve-mediated transport pathway also plays a key role in the distribution of intranasally administered drugs to brain areas distant from the olfactory bulbs [31]. Experimentally, it is difficult to determine the contributions, individually, from different pathways into the CNS after intranasal administration.

4. Biofate of neurotherapeutics

4.1 Drug absorption

The first step in absorption of drugs from the nasal cavity is the channeling through mucus. Small and uncharged molecules easily find their way while passing through this layer, whereas large and charged particles find it more difficult to cross. Mucin, a principle protein in mucus, has potential to bind solutes and hinder diffusion. Structural changes may occur in the mucus layer due to environmental factors such as a change in pH or temperature. The foremost obstacles to drug absorption across nasal mucosa are potential metabolism in the nasal cavity before reaching the target site and limited residence time in nasal cavity [32]. Various studies indicate that drug concentrations in CSF increases with an increase in lipophilicity or partition coefficient of drugs, concluding partition coefficient as a major factor governing nasal drug absorption. It was observed that a quantitative relationship exists between partition coefficient and nasal drug absorption [33]. The permeability at the site where the formulation is deposited and the area of the nasal cavity exposed also affects nasal absorption of drugs. Absorption and permeability of drugs across nasal mucosa is affected by various factors as illustrated in Figure 6 [34].

4.2 Drug distribution

Efficiency of nasal absorption is primarily affected by drug distribution in the nasal cavity. The mode of drug administration may affect the distribution pattern of drug in the nasal cavity, which in turn can help determine the extent of drug absorption. Nasal deposition of particles is related to resistance to airflow provided by the nasal mucosa of an individual. With nasal breathing, almost all particles with an aerodynamic particle size in the range of 10 – 20 µm are deposited on the nasal mucosa. The deposition pattern of particles in the respiratory tract is a function of particle size and respiratory patterns. The shape, density and hygroscopicity of particles,
and the pathological conditions in the nasal passage influence the deposition of particles, whereas particle size distribution determines the site of deposition and affects the subsequent biological responses in animals and humans. Furthermore, improvement in drug delivery systems and drug delivery formulations, through the use of integrated drug delivery devices, is necessary to achieve an improved clinical outcome and better patient compliance [35-37].

Particle deposition in the respiratory tract is usually assessed by three mechanisms: inertia, sedimentation, and diffusion. Among these, the first mechanism is the chief mechanism in nasal deposition. Any particle with an aerodynamic diameter of 50 µm or greater does not enter the nasal passage. The site of drug deposition within the nasal cavity depends on the type of drug delivery system and the technique of drug administration used [38,39].

5. Strategies to enhance direct nose to brain drug delivery

Surface engineering of drug carrier would serve as one of the excellent approaches to manage drug delivery properties of formulations by interaction of surface coating with a biological system. This strategy could potentially dictate the utility of this drug delivery route so that it would be more successful. This section highlights the key studies concerning surface modification of drug delivery/carrier systems to enhance direct nose to brain drug delivery.

5.1 Chitosan surface modification

Chitosan, an alkaline hydrolytic derivative of chitin, has a better solubility profile, less crystallinity and is promising for chemical modifications due to presence of various reactive functional groups such as hydroxyl, acetamido and amine. The chemical modification of chitosan is of great interest because the modification would not change the fundamental skeleton of chitosan, would keep the original physicochemical and biochemical properties and finally would bring new or improved properties. This is the prime reason behind the extensive interest of researchers for the utilization of chitosan in pharmaceutical and biomedical applications. Various chemical modifications have been carried out on chitosan, including oligomerization, alkylation, acylation, quaternization, hydroxyalkylation, carboxyalkylation, thiolation, sulfation, phosphorylation, enzymatic modifications and graft copolymerization. The chemical modification affords a wide range of derivatives with modified properties for specific applications in diversified areas mainly of pharmaceutical, biomedical and biotechnological fields [40].

Mistry studied the effect of chitosan coating on in vitro uptake and transport of 100 nm polystyrene (PS) nanoparticles over porcine olfactory epithelium mounted in Franz’s
diffusion cell. It was found that PS nanoparticles surface-modified with chitosan were retained in greater numbers in the mucus layer compared with unmodified equivalents [41]. They also observed that increasing the cationic charge on chitosan-modified particles, by reducing the pH of the buffer from pH 6.0 to 4.5 in porcine model, increased the particle association with mucus from 10 ± 3% to 39 ± 4% of administered dose. This demonstrated that the mucoadhesive potential was primarily controlled by electrostatic interactions between mucus and chitosan-coated nanoparticles.

Kumar et al. [12] studied, in rodents, the direct nose to brain transport of small molecular weight drug (risperidone) in simple nanoemulsion and chitosan-modified nanoemulsion formulation. It was found that the highest concentration (78%) of risperidone in the brain was obtained with a chitosan-modified mucoadhesive nanoemulsion formulation, compared with a simple nanoemulsion formulation (57%) and simple risperidone solution (62%). The mechanism of drug transport was not discussed in detail.

The enhanced direct nose to brain drug delivery effect of chitosan formulations is suggested to be attributable to a combination of: i) the passive targeting ability of chitosan by mucoadhesion resulting in increased residence time of formulation over the olfactory region and ii) the increased permeability of 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 for nose to brain delivery than polymeric nanoparticles. One may suggest that a nanoemulsion formulation is less likely to commence oxidative stress through mitochondrial disruption as it does not contain solid surfaces. Such solid surfaces may mechanically disrupt mitochondrial membranes and cristae as may be the case for polymeric nanoparticles [42].

5.2 PEG surface modification
Surface modifications with polyethylene glycol (PEG) add new physicochemical properties to existing polymers, thereby overcoming limitations associated with them, especially regarding their solubility [43]. Lai et al. in their studies found that conjugating a 2 kDa homopolymer PEG to the surface of 100 and 200 nm PS nanoparticles, diffusion coefficient of nanoparticles through human cervicovaginal mucus increased by 20 and 381 times, respectively [44]. This is potentially relevant because a low PEG molecular weight and a high PEG surface coverage were required for rapid penetration of mucus [45]. Furthermore, PEG was adsorbed onto the PS surface rather than covalently attached and consequently may have been desorbed or displaced from the nanoparticle surface in 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 fibers to reduce transmucosal movement of nanoparticles) and nanoparticle core composition (which affects particle surface charge) and effect of proteins adsorbed on nanoparticle surface from biological milieu [45,46].

5.3 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 abundantly in nature and can recognize sugar residues on biological surfaces. Their selective affinity for biological surfaces may be useful for direct nose to brain drug delivery.

In a recent study, wheat germ agglutinin (WGA) was conjugated to coumarin-loaded poly(ethylene glycol)-poly(lactic acid) (PEG–PLA) nanoparticles (d_{avg}: 85 – 90 nm) and administered intranasally in rats. WGA binds to N-acetyl-D-glucosamine and sialic acid residues both of which are abundant on the nasal epithelial membrane. A twofold increase in coumarin was observed in olfactory bulb, olfactory tract, cerebrum and cerebellum within 15 h of a single dose of lectin-modified nanoparticles compared with unmodified nanoparticles, without any evidence of ciliotoxicity [47,48].

Gao et al. investigated an intranasal delivery system for vasoactive intestinal peptide (VIP; MW 3326), a neuroprotective peptide, administered in PEG–PLA and in WGA-coupled PEG–PLA nanoparticle formulations. VIP concentrations were found to increase in the olfactory bulb, cerebrum and cerebellum by 3.57-, 3.63- and 4.74-fold, respectively, when compared with intranasal solution of VIP; these values increased further to 5.66-, 6.61- and 7.74-fold for VIP-loaded WGA-PEG–PLA nanoparticles. The increase in VIP concentrations also corresponded to improved memory function, as determined by water maze behavioral 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 with control animals. The in vivo nasal ciliotoxicity studies of these prepared formulations, in rats, suggested that there is no visible change in morphology and integrity of the nasal cilia [49]. Liu et al. evaluated the in vivo toxicity and immunogenicity of WGA-PEG–PLA nanoparticles after repeated intranasal administration to Sprague-Dawley rats. WGA-PEG–PLA nanoparticles induced slight excitotoxicity and oxidative stress, as evidenced by increased glutamate levels in rat brain and increased lactate dehydrogenase activity in rat olfactory bulb. This slight toxicity demonstrated that WGA-PEG–PLA nanoparticle is a safe carrier system for the treatment of CNS diseases via intranasal administration [50].

5.4 Lipid surface modification
Patel et al. [51] reported the formulation and evaluation of risperidone-loaded solid lipid nanoparticles (SLNs) for brain targeting and their findings substantiate the existence of a direct nose to brain route for nanoparticles administered to the nasal cavity. However, modification of the lipid matrix
of lipid-based nanoparticle formulations may improve the formulation characteristics. Recently, Pardeshi et al. [52] prepared surface-engineered SLNs for brain targeting. They assessed the influence of stearylamine, a surface charge-modifier, on particle size, ζ-potential and stability of SLNs. Stearylamine induces positive charge on the surface of nanoparticles and also contributes to increased stability. Stearylamine serves as an electrostatic stabilizer by maintaining ζ-potential of fabricated system to the optimum. Also, ropinirole-loaded surface-modified SLNs had shown enhanced therapeutic efficacy in terms of ability to reduce tremors in a tremor-induced rat model of Parkinson’s disease (PD), when compared with marketed tablet formulation of the same drug. Thus, lipid surface modification may contribute to the development of products with improved formulation and stability characteristics, which would serve as a promising approach for brain targeting via intranasal administration of neurotherapeutics.

5.5 Peptide surface modification
A key mechanism to enhance nasal adsorption of nanoparticles is to improve their transmucosal transport, which may be facilitated by surface modification with bioactive peptides such as cell-penetrating peptides (CPPs). Among the CPPs, low molecular weight protamine (LMWP) is potent in mediating cellular translocation of the attached cargos. LMWPs are neither antigenic nor mutagenic and exhibit a much lower toxicity, and thus have an improved safety profile over protamine. Xia et al. suggested that LMWP may serve as an effective and safe CPP for facilitating nose to brain delivery of drug-loaded nanoparticles. To justify this hypothesis, LMWP was functionalized to the surface of PEG-PLA nanoparticles (via a maleimide-mediated covalent binding). The brain targeting ability of the developed nanoparticles was extensively studied following intranasal administration, employing coumarin-6 as a probe. Cellular experiments have shown that, LMWP-functionalized nanoparticles exhibited significantly enhanced cellular accumulation (in 16HBE14o cells as cell model), as compared with unmodified nanoparticles via both lipid raft-mediated endocytosis and direct translocation process without showing any observable cytotoxicity. Results of brain distribution studies clearly indicated that after intranasal administration, LMWP-functionalized nanoparticles could be effectively delivered to the CNS along both olfactory and trigeminal nerve pathways [53].

6. Applications

6.1 Delivery of macromolecules to CNS
In today’s scenario of advanced proteins, peptides and vaccine research, intranasal administration of such macromolecular compounds provides an excellent platform for direct delivery to the CNS. Large molecular size and susceptibility to enzymatic degradation is the prime reason behind low bioavailability of such compounds. Proteins and peptides are generally administered parenterally owing to their physicochemical instability and susceptibility to hepatogastrointestinal first-pass elimination. On this background, intranasal administration seems to be a promising option [54].

Delivery of protein therapeutic agents to the CNS involves extraneuronal transport as it occurs within minutes rather than hours. A number of protein therapeutic agents have been delivered to the CNS through the intranasal delivery route. Neuropotropic factors such as nerve growth factor (NGF) [55], IGF-I [56], fibroblast growth factor (FGF), and ADNF-12 [57], growth differentiation factor 5 (GDF-5) [58] have been intranasally delivered to the CNS in rodents. Studies in humans, with proteins such as arginin-vasopressin (AVP) [59], cholecystokinin (CCK) analog [60], adrenocorticotrophic hormone (ACTH) [61] and insulin [62,63] have revealed that they can be delivered to the brain directly from the nasal cavity. Gozes et al. have shown that intranasal administration of a VIP analog (containing 28 amino acids) prevented learning and memory impairments resulting from cholinergic blockade in rats treated with aziridinium [64].

Wolf et al. reported, for the first time, that therapeutic level of a lysosomal enzyme, recombinant iduronidase (IDUA, MW: 82 kDa), can bypass the BBB and can be delivered to adult human brain following intranasal administration. This novel strategy could potentially be used to treat CNS manifestations of multiple lysosomal storage diseases [65].

Intranasal delivery also opens the possibility for treatment of diseases that are known to derive from dysfunctions in central nervous neuropeptide signaling such as Alzheimer’s disease (AD) and obesity. Hallsschmid et al. investigated the therapeutic potential of intranasal neuropeptide administration in metabolic disorder and obesity. Intranasal administration of melanocortin4,10 (MSH/ACTH4-10), a melanocortin receptor agonist, enables the modulation of central nervous signaling pathways of body weight regulation by inducing weight loss in normal-weight humans [66]. Dhuria et al. proposed the phenylephrine incorporated formulations containing therapeutic neuropeptides hypocretin-1 (HC) and L-Tyr-D-Arg (D-KTP). Intranasal administration showed a threefold increase in deposition of both neuropeptides onto the olfactory epithelium, suggesting the feasibility of effective nose to brain neuropeptide delivery [67].

Impaired brain insulin signaling may result in cognitive decline in patients with memory disorders like AD. Insulin can be effectively delivered directly to the brain via the intranasal route that enables this hormone to bypass the BBB and modulate CNS functions [68]. Clinical studies in both healthy human subjects and in patients with cognitive deficits as in AD, demonstrated the direct action of prolonged intranasal administration of insulin on brain functions, improving memory and mood in the absence of systemic side effects [69-71].

6.2 Delivery of DNA plasmids to CNS
Among several routes available for immunization, the nasal route has its own worth because of its ease of administration and induction of potent immune responses, particularly in
the respiratory tract. However, adjuvants and delivery systems are required to enhance immune responses following nasal immunization [22]. The use of poly(lactide-co-glycolide) (PLGA) microparticles as adjuvants and delivery systems for proteins and DNA vaccines for nasal immunization was reviewed by Vajdy and O’Hagan [72]. It has also been reported that after nasal administration of DNA plasmids, the level of plasmid in brain was 3.9 – 4.8 times higher than the plasmid concentration in lungs and spleen. It was also found that the plasmid DNA reached the brain within 15 min following intranasal administration [73]. This indicates that nasal administration could be a potential route for delivery of therapeutic genes to the brain with reduced side effects in other organs.

6.3 Delivery of small molecules to CNS
Many small molecules have been shown to be transported directly to the brain and/or CSF from the nasal cavity. The properties of small molecules such as size, and lipophilicity affect their delivery to CNS following intranasal administration. A comparison of brain olfactory bulb concentrations achieved 30 min after intranasal administration of 7.4 nmol dopamine (153 Da) with those obtained after intranasal administration of 7.4 nmol NGF (26,500 Da) to rats revealed a fivefold higher delivery of low molecular weight dopamine to the brain [74]. Li et al. developed an ethyl laurate-based microemulsion formulation for direct nose to brain delivery of diazepam and found rapid onset of action attained within 2 – 3 min after administration, with the bioavailability of about 50% after nasal spray compared with intravenous injection. Results clearly indicated that the developed system could be efficiently utilized for effective delivery to the brain after intranasal administration during emergency treatment of status epilepticus [75].

6.4 Delivery of stem cells
The safety and efficacy of cell-based therapies for neurological diseases depend on the mode of cell administration. Stem cells have the ability to self-renew and differentiate into other types of cells [76]. Cell replacement or gene transfer to the diseased or injured brain have provided a basis for development of potentially powerful new therapeutic strategies for a broad spectrum of human neurological diseases including stroke, cerebral vascular diseases, traumatic injury, immune system-mediated diseases, multiple sclerosis, psychiatric disorders, epilepsy and neurodegenerative diseases like PD and AD. Over the last two decades, stem cell technologies have become an increasingly attractive option to investigate and treat brain diseases. Stem cells may treat neurological disorders by means of cell replacement (replacement of dead or damaged neurons) or production of neurotrophic factors to support host neurons [77,78].

The major sources of stem cells that are currently being studied for potential therapeutic application in neurodegenerative diseases include pluripotent sources such as embryonic stem cells (ESCs) derived from the inner mass of embryos, embryonic germ cells (EGCs) derived from the gonadal ridge of the fetus as well as lineage-restricted sources such as neural stem cells (NSCs) derived from fetal, neonatal or adult brain. NSCs are capable of self-renewal and can give rise to several neuroepithelial-derived brain cell types: neurons, astrocytes and oligodendrocytes. Brain-derived NSCs have received wide attention as a source of cells for neural cell replacement and serve as an attractive therapeutic paradigm [79]. Stem cells have several characteristics which make them suitable for replacement of the nervous system: i) the ability to self-renew and differentiate into new neurons or glial cells, ii) easy propagation which allows genetic manipulations, iii) a high tropism for tissues affected by inflammation or by malignancies, which make them the ideal vehicle for delivery of beneficial proteins, iv) the possibility to integrate into the host brain [80].

Danielyan et al. demonstrated the therapeutic efficacy of intranasally delivered mesenchymal stem cells (MSCs) to the brains of 6-hydroxydopamine-lesioned rat model of PD. Intranasal application of MSCs resulted in appearance of cells in olfactory bulb, cortex, hippocampus, striatum, cerebellum, brainstem and spinal cord. Results suggested that intranasal administration provides a safe, effective and highly promising non-invasive alternative to traumatic surgical procedures of transplantation and allows targeted delivery of stem cells to the brain [81].

7. Drug molecules and carrier systems being delivered by direct nose to brain transport pathway
Multiparticles, including micro- and nano-dimensional drug delivery vehicles have excellent and broad prospects in pharmaceutical field. Scientists from various disciplines have been harnessed by the superior outcomes obtained from such carrier systems viz. greater therapeutic efficacy with reduced dosing frequency. A variety of therapeutic agents loaded in various carrier systems have been reported to be delivered to the brain via the intranasal route. A brief summary of drug molecules, proteins, peptides, hormone and/or biological cells like stem cells and respective drug carrier systems being delivered by direct nose to brain drug delivery route is presented in Table 2.

8. Patents on nose to brain drug delivery systems
Despite the fact that many of them have not reached the market so far, a good number of patents have been received on various drug molecules loaded in a variety of carriers for direct nose to brain drug delivery. Due to the challenges of large-scale commercial production, only few patents have been transferred into commercial marketed products. Some patents
related to direct nose to brain drug delivery systems are illustrated in Table 3.

### 9. Future perspectives

In the last few decades, the attention of various research groups has shifted to the development of novel drug delivery systems to circumvent the BBB. This is due to the significant challenges faced by researchers, academicians and industrialists looking at effective treatment strategies for increasing incidence of brain disorders in the elderly population. Present review embodies the fact that many of the drug delivery systems like polymeric micro- and nanoparticles, nanoemulsions, polymeric micelles, liposomes, etc. are potential carriers for delivery of drugs across the BBB for treatment of CNS disorders. However, there are still a stack of challenges, as most of the potent CNS acting drugs are hydrophilic in nature which makes it difficult for them to cross the BBB. Surface modification of drug delivery carriers serves as one of the promising approaches to circumvent this budding problem. Considering the potential benefits of nasal drug delivery systems (patient compliance and risk-benefit ratio), utilization of this non-invasive method of drug delivery offers a potential alternative to invasive methods and could be exploited, in the near future, for development of novel drug delivery systems. No doubt, this direct nose to brain drug delivery system would have a bright future in the pharmaceutical industry and would definitely bring a large number of commercial products to the pharmaceutical market in near future.

### 10. Conclusion

A successful drug delivery system is one which offers commercial applicability to pharmaceutical industries for large-scale production. CNS drug delivery is complex due to limitations imposed by the BBB. Direct nose to brain drug delivery system is a potential strategy to overcome the obstacles presented by the BBB. Intranasal delivery bypasses the BBB to target CNS, reducing systemic exposure of drug, thereby reducing the systemic side effects. It is an attractive option of drug delivery due to its non-invasiveness. A variety of neurotherapeutic agents including small drug molecules, proteins, peptides, hormones and biological cells such as stem cells can be delivered by this route, thereby yielding new insights into prevention and management of different neurological disorders. It is uncertain, however, whether the drug is being released from the carrier system in the nasal cavity and transported to CNS, or the carrier system is transported along olfactory and/or trigeminal nerve pathways into the CNS where the drug is released. Thus, more basic research is required to determine the possible transport pathway of therapeutic carrier to the CNS and their further fate into the biological system. Again, delivery of surface engineered carrier systems through passive or active targeting approach would be desirable for further progress in the field.

### 11. Expert opinion

A major hurdle in drug delivery to the brain is the presence of the BBB which restricts the diffusion of drugs from systemic...
circulation into the CNS even if it is disrupted in certain pathological conditions. Although the BBB, together with enzymes, serves to shield the brain and spinal cord from a variety of pathogens and toxicants, it also presents a significant obstruction to many of the xenobiotics, particularly of polar and large molecular weight drugs such as proteins and peptides, in the treatment of CNS disorders. This could be the reason that many neurotherapeutic agents failed to stay on the pharmaceutical market because of their inability to achieve sufficient levels in brain through systemic circulation. Intranasal administration offers a practical, safe, convenient and non-invasive alternative to various conventional and invasive drug delivery techniques, listed earlier elsewhere (Section 1), as a transport pathway for direct delivery of drugs effectively to the CNS, bypassing the BBB. Thus, nose to brain delivery of drugs have been tried by several researchers around the globe to utilize and explore the advantages offered by this route. Nose to brain delivery is most likely mediated by olfactory and/or trigeminal nerve pathways. Neuroepithelium is the only part of CNS that is directly exposed to external environment through the nasal cavity. Thus, better target specificity can be achieved due to direct movement of drug from the submucosal space of the nose into the CSF compartment of brain.

Although the precise mechanisms behind CNS targeting via intranasal administration is not entirely understood yet, many of the reported evidence demonstrates that integrated nerve pathways (olfactory and/or trigeminal nerve pathways) connecting nasal passages to brain and spinal cord, along with the pathways involving CSF and lymphatic systems, have been implicated in target-specific delivery of molecules from nasal cavity to the CNS. A combination of these pathways is responsible, although one pathway may predominate. In the last few decades, major reports have emerged on the development of novel drug delivery systems to circumvent the BBB. This is due to the significant challenges faced by researchers, academicians and industrialists looking for effective treatment strategies for the increasing incidence of brain disorders in elderly population. There are still a number of challenges that remain, as most of the potent CNS acting drugs are hydrophilic in nature which makes it difficult for them to cross the BBB. From the available research literature, it has been concluded that surface modification of drug-carrier system would serve as one of the excellent approaches to circumvent this budding problem through enhanced direct nose to brain drug delivery. Considering the potential benefits of nasal drug delivery systems (patient compliance and risk–benefit ratio), utilization of this non-invasive method of drug delivery offers a potential alternative to various invasive methods and could be exploited in near future for development of novel drug delivery systems.

### Table 3. Patents on nose to brain drug delivery systems.

<table>
<thead>
<tr>
<th>Drug</th>
<th>Therapeutic application</th>
<th>Drug delivery system</th>
<th>Patent</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>Brain disorders</td>
<td>Solution</td>
<td>Frey US2001631093B1 (2001)</td>
<td>[83]</td>
</tr>
<tr>
<td>Therapeutic cells</td>
<td>Neurodegenerative</td>
<td>–</td>
<td>Frey et al. US201282316082 (2012)</td>
<td>[84]</td>
</tr>
<tr>
<td>NMDA receptor antagonist</td>
<td>PD and AD</td>
<td>Extended-release dosage form</td>
<td>Meyerson et al. US20050245617A1 (2005)</td>
<td>[89]</td>
</tr>
<tr>
<td>Proteasomes glatiramer</td>
<td>Neurodegenerative</td>
<td>Nanoemulsion</td>
<td>Meyerson et al. US20060240043 (2006)</td>
<td>[90]</td>
</tr>
<tr>
<td>Diazepam</td>
<td>Epilepsy</td>
<td>Microemulsion</td>
<td>Levin US20050281751A1 (2005)</td>
<td>[91]</td>
</tr>
</tbody>
</table>

AD: Alzheimer’s disease; NMDA: N-methyl-o-aspartate; PD: Parkinson’s disease. Modified from [102].
Recent reports have demonstrated that a direct nose to brain drug delivery system bypasses the BBB to target the CNS, reducing systemic exposure of drug, thereby reducing the systemic side effects. However, this route needs more research into the safe trafficking of neurotherapeutics to the brain and their further fate in biological system. Again, estimation of effect of different surface modifications of carrier systems and introduction of specific ligand would be desirable for further progress in the field.

Declaration of interest

The authors state no conflict of interest and have received no payment in preparation of this manuscript.

Bibliography

Papers of special note have been highlighted as either of interest (●) or of considerable interest (★★) to readers.

1. Pardeshi CV, Rajput PV, Belgamwar VS, Tekade AR. Formulation, optimization and evaluation of spray-dried mucoadhesive microspheres as intranasal carriers for Valsartan. J Microencapsul 2011;29:103-14


★★ A good review on nasal drug delivery system highlighting factors affecting nasal drug absorption and applications.


★★ An excellent review which focus on various strategies for effective brain targeting.


★★ A nice experiment which demonstrates the direct nose to brain delivery of olanzapine for effective treatment of CNS disorder like schizophrenia.


★★ A nice study which illustrates the direct nose to brain delivery of hydrophilic antiparkinsonian drug ropinirole hydrochloride.


• An excellent overview on direct nose to brain delivery of nanoparticulate carriers and strategies to enhance their brain targeting.


• A good review focusing challenges and solutions in nasal drug delivery systems.


★★ A nice review highlighting the major applications of direct nose to brain delivery.


★★ An excellent review on mechanisms and pathways of drug transport to brain after intranasal administration.

25. Thorne RG, Pronk GJ, Padmanabhan V, Frey WH. Delivery of insulin-like growth factor-I to the rat brain and
spinal cord along olfactory and trigeminal pathways following intranasal administration. Neuroscience 2004;127:481-96


** An excellent discussion on nasal mucoadhesive in situ gelling drug delivery system.


65. Wolf DA, Hanson LR, Aronovich EL, et al. Lysosomal enzyme can bypass the blood-brain barrier and reach the CNS following intranasal administration. Mol Genet Metab 2012;106:131-4


82. Frey WH II, Danielyan L, Christoph H. Methods, pharmaceutical compositions and articles of manufacture for administering therapeutic cells to the animal central nervous system. US8283160B2; 2012

83. Frey WH II. Administration of neurotrophic agents to the central nervous system. EP 1137401 B1; 2005

84. Frey WH II, Danielyan L, Christoph H. Methods, pharmaceutical compositions and articles of manufacture for administering therapeutic cells to the animal central nervous system. US8283160B2; 2012

85. Frey WH II. Method for administering neurotrophic agents to the central nervous system. US6180603B1; 2000


91. Levink B. Directed intranasal administration of pharmaceutical agents. US0281751A1; 2005


** An excellent publication on delivery of biologics to the CNS after intranasal administration.


96. Frey WH II, Danielyan L, Christoph H. Methods, pharmaceutical compositions and articles of manufacture for administering therapeutic cells to the animal central nervous system. US8283160B2; 2012

97. Frey WH II. Administration of neurotrophic agents to the central nervous system. EP 1137401 B1; 2005

98. Frey WH II, Danielyan L, Christoph H. Methods, pharmaceutical compositions and articles of manufacture for administering therapeutic cells to the animal central nervous system. US8283160B2; 2012


101. Levink B. Directed intranasal administration of pharmaceutical agents. US0281751A1; 2005

102. Went GT, Fultz TJ. Methods and compositions for the treatment of
CNS-related conditions. US0252788A1; 2006


94. Choi YM, Kim KH. Transnasal microemulsions containing diazepam. US0002987A1; 2005


100. Wermeling DP. System and method for intranasal administration of lorazepam. US0055571A1; 2001


** A nice review discussing the novel approaches for CNS drug delivery, intranasal drug carriers and patents thereon.

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