Nose-to-Brain Drug Delivery: Novel Approaches for New Drug Development

Kajal Deshmukh[1] and Charitra Nagarkatti[1]
Bachelor of Pharmacy
Saraswathi Vidya Bhavan's College of Pharmacy, Thane, Maharashtra, India.

Sunanda Patil[2]
Department of Pharmacology, Assistant professor
Saraswathi Vidya Bhavan's College of Pharmacy, Thane, Maharashtra, India.

Abstract

In the current scenario, delivery of drugs to the brain is a very challenging approach due to the presence of physiological barriers like BBB (Blood Brain Barrier) that prevents the delivery of drugs from reaching the CNS. Thus, the intranasal route has drawn the attention of scientists as a convenient, non-invasive, and safe route to increase the bioavailability of drugs targeted to the brain. The underlying mechanism of the nose-to-brain drug delivery is still unknown;hence, it becomes essential to investigate the exact mechanism. The literature for this review is compiled by reviewing various articles from databases like PubMed, Scopus, MedLine, and ScienceDirect. This review provides insights into the drug transport pathways, delivery systems, various approaches, and also future perspectives related to the nose-to-brain drug delivery system which elucidates the novel intranasal approach as an emerging and promising alternative route for direct nose-to-brain drug transport.

Keywords: Blood-brain barrier (BBB); brain targeting; intranasal; central nervous system (CNS); nose-to-brain; nanoparticles; nasal devices.

Introduction

There are various approaches for nose-to-brain drug delivery which transports drugs to the brain through the intranasal route with the help of nasal devices to circumvent the problems faced by this route. Despite owning so many benefits, the intranasal route faces a lot of challenges. The BBB(Blood-Brain Barrier), being the most complex challenge for drug delivery, is a highly selective, semi-permeable barrier that regulates the transport or exchange of molecules between the CNS(Central Nervous System),and the circulating blood. Thisbarrier acts as a shield that protects CNS from pernicious substances, pathogens and provides nutrients to CNS such as glucose, amino acids, water, etc., which are essential for neural function. BBB comprises a complex system consisting of endothelial cells of brain microvasculature, astrocyte end-feet (astroglia) that surrounds the brain capillaries, pericytes, perivascular mast cells, immune cells, neurons, microglia, oligodendrocytes, and tight junctions.[1,2] These all together form a neurovascular unit as shown in fig.1. BBB is localized at the level of endothelial cells and separates brain interstitial fluid (ISF) from the blood, whereas the blood-cerebrospinal fluid barrier (BCSFB) separates CSF from the choroid plexus interstitial fluid (CPISF). Dueto the endothelial cells and the tight junctions between them, BBB and BCSFB collectively prevent the paracellular or passive diffusion of most substances into the brain tissue.[3-6] Hence this is the rate-determining step in the absorption of the drug molecules in the brain. Highly lipid-soluble drugs can passively cross the BBB, while glucose, amino acids, and other foreign molecules transport actively into the cerebrum.

Methods

The literature for this review is compiled by reviewing various articles from databases like PubMed, Scopus, MedLine, and ScienceDirect.

Pericytes

Pericytes are the specialized cells that play an important role in maintaining the functions of BBB such as homeostasis, angiogenesis as well as contractile, immune, and phagocytic functions. They are multipotent cells and hence find great potential for therapy.[7] The presence of special cells called astrocytes and pericytes, which are the components of supporting tissue at the base of the endothelial membrane, form a solid envelope surrounding the capillaries. As a result, the intercellular (paracellular) passage gets blocked and for a drug to gain access into the brain from the capillary circulation, it has to pass through the cells (transcellular) rather than between them.[8,9] A drug may thus gain access to the brain only via one of the two pathways:

1. Passive diffusion through the lipoidal barrier (restricted to the small molecules having high o/w partition coefficients).
2. Active transport of essentials nutrients such as sugars and amino acids. The selective permeability of lipid-soluble moieties through the BBB makes an appropriate choice of drug to treat CNS disorders, an essential part of therapy.

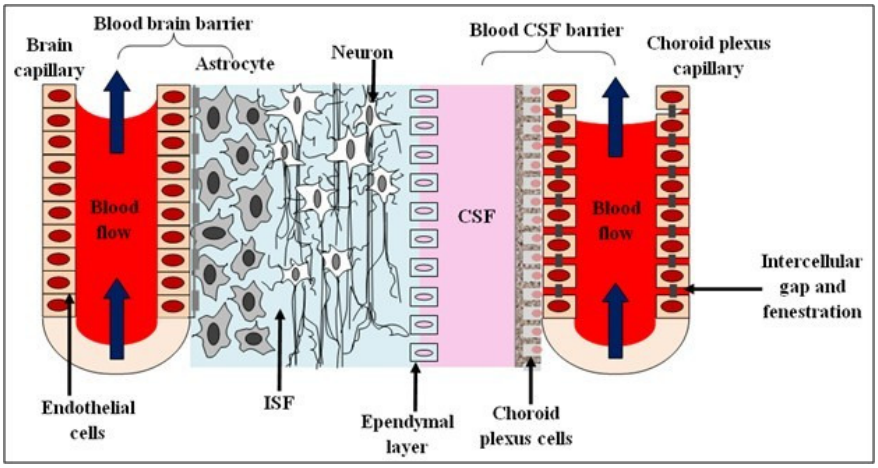

Fig.1 Diagrammatic representation of the barriers present in the brain. Blood-Brain barrier(BBB) and Blood-Cerebrospinal fluid barrier collectively protect the brain from harmful substances. The endothelial cells of BBB, astroglia, pericytes, neurons, microglia, and tight junctions constitute a neurovascular unit. Recreated from Pavan B, Dalpiaz A, Ciliberti N, Biondi C, Manfredini S, Vertuani S. Progress in drug delivery to the central nervous system by the prodrug approach. Molecules. 2008 May 1;13(5):1035-65. Doi: 10.3390/molecules13051035. PMID: 18560328; PMCID: PMC6245073.

Characteristics of a Drug Molecule

Lipophilicity is not the only criteria to cross BBB but also there are many other characteristics that a drug molecule must possess such as the surface activity of the molecule, relative size, permeability, surface area, hydrophobicity of molecule.[10,11]

Problems to cross BBB

There occur many problems when it comes to the absorption of drug molecules into the brain. Some of them are mentioned below:

- Inactivation of drugs by the enzymes present in the brain.
- Low molecular weight drugs having a size less than 700 Daltons can bind to protein in the plasma furthermore impeding its transportation into the brain.[12]
- The efflux transporters present in the vasculature of BBB throw away the drug molecules that anyhow gain entry into the brain by active transport. These transporters can identify a wide range of compounds that leads to drug resistance.[13]

Different Approaches of Drugs to cross BBB[14,15]

- ***Invasive techniques:*** Includes osmotic disruption of BBB, focused ultrasound technology, intracerebro-ventricular and intrathecal infusions, interstitial wafers, microchips, etc.
- ***Non-invasive techniques:*** Includes cell-based delivery, Trojan horse approach, use of chimeric peptides, intranasal delivery, etc.

Drug delivery to the brain via nasal route involves many techniques or mechanisms such as polymersomes, receptor-mediated, carrier-mediated, nanoparticle, prodrug, bioconversion strategies, peptide masking, cationic transport, etc.

Nasal Anatomy and Route of Drug Delivery to the Brain

It consists of three main parts:

- Vestibular region – anterior external opening.
- Respiratory region – consists of ciliated and non-ciliated columnar cells, goblet cells, and basal cells.
- Olfactory region – consists of olfactory receptor cells, basal and sustentacular cells.[16]

Drug delivery through the nasal route is mainly associated with the olfactory area and the pathways related to the transfer of drugs directly to the brain via this route.[17] There are three different pathways after nasal administration. Once the drug gets administered, it undergoes three pathways: olfactory, trigeminal, or systemic pathway. Another mechanism through which drugs can cross neuronal membrane is paracellular or transcellular transport. These pathways ultimately reach the brain bypassing the BBB.

Transport of drugs via nasal route directly to the brain:

Intranasal drug delivery is one of the non-invasive methods used to deliver drugs to the brain. It involves the olfactory and the trigeminal nerve system that bypasses the BBB. Hence it is widely used to treat various neurodegenerative diseases.[18] It needs special devices to administer drugs to the nasal epithelium. There are three different mechanisms involved in this transport:

1. Systemic pathway (minor route)
2. Neuronal pathways (primary routes)
   - Olfactory pathway
   - Trigeminal pathway

1. Systemic pathway

As mentioned earlier, a fraction of the drug gets absorbed into the systemic circulation. That is because of the highly vascularized respiratory epithelium that comprises continuous and fenestrated endothelium. There arises a risk of drug entering into systemic circulation that ultimately alters the amount of drug reaching the brain and impedes its therapeutic effects. It occurs because it allows the passage of small and large molecules as well into the blood circulation. That leads to several side effects but the presence of nasal vasculature having a tight junction prevent the entry of drug molecules into the systemic circulation. Except for the low molecular weight, lipophilic molecules or drugs can enter the systemic circulation.[19]

2. Neuronal pathway

- *Olfactory pathway:* Once the drug gets administered, it concentrates in the olfactory or respiratory epithelium that gets absorbed and reaches the olfactory bulb or CSF via olfactory sensory neurons.[20,21] The pathway followed by the drug is either transcellular or paracellular from the olfactory epithelium. From the CSF, the drug enters by diffusing into ISF in the brain.[19,22] Once the drug gains entry into the olfactory bulb, it follows two pathways:-
  i. Intraneuronal pathway– It takes hours to days to reach the brain. Its mechanism is adsorptive, receptor-mediated, or endocytosis.
  ii. Extraneuronal pathway– Reaches the brain within a minute. Its mechanism is bulk transport through perineuronal channels.
- *Trigeminal pathway:* This pathway involves the trigeminal nerve that is the fifth and the largest cranial nerve. Drugs traversing this path undergo intracellular transport (axonal transport) or endocytosis to reach the brain stem.[19,22] The trigeminal nerve comprises three branches:
  i. Ophthalmic – Innervates dorsal part of the nasal mucosa and anterior nose
  ii. Maxillary – Innervates turbinates of the nasal mucosa
  iii. Mandibular

Neurons from the ophthalmic and the maxillary branches pass directly through the nasal mucosa and play a vital role in the drug delivery from nose-to-brain. The mechanism of drug delivery via trigeminal pathway involves several transports such as paracellular, transcellular, carrier-mediated, receptor-mediated and transcytosis. A part of the trigeminal nerve crosses cribriform plate entering the spinal cord, medulla and pons, thus paving another pathway of drug delivery via nasal route.

Mucociliary Clearance (MCC)

Goblet cells of the respiratory epithelium secrete mucus. It forms a mucus layer that entraps many particulate matters or harmful substances entering the nose and preventing them from entering the lungs[23]. Such substances get adhered to or dissolved in the mucus layer are then transported to the nasopharynx. Later these substances are discharged into GIT. This layer is renewed every 15 to 20 minutes that results in particle clearance of the same. This mucosal clearance with adsorbed or dissolved particulate substances into GIT is called Mucociliary clearance.[24,25]

Delivery Systems used in the Nose-to-brain Drug Delivery

1. Polymersomes-based Drug Delivery:

These are tiny hollow spheres composed of lipid bilayer membranes with an aqueous core similar to liposomes.[26]

Pathogenesis:

There are extracellular vesicles (EVs) called exosomes released by every eukaryotic cell found everywhere in the body. They are also known as intercellular messengers as they transfer payloads of peptides, proteins, lipids, and nucleic acids between the donor and recipient cell in the physiological states.[27,28]

Formerly, exosomes were considered as a disposal mechanism of cells during the maturation process, but under certain pathological conditions, especially in proteinopathies, including Alzheimer's disease(AD), Parkinson's disease(PD), Amyotrophic Lateral Sclerosis(ALS), and Frontotemporal dementia(FTD) there is increased use of this disposal mechanism as the other cellular mechanisms such as proteasome and autophagy-lysosome system fail in eliminating the accumulated amyloidogenic proteins mainly responsible for disease progression.[29,30] These exosomes are lipophilic that can cross BBB and come into the blood. These can get detected in the blood analysis report.[31] Hence, there is a substantial scope as a source of the biomarker in diagnosing patients at an early disease stage, monitoring the disease states and the progression of the same.[32] Their ability to cross the BBB in the opposite direction can make them useful in delivering drugs and biomolecules into the brain.[33]

Neuroinflammation may be a primary pathophysiological feature shared by both acute and chronic neurodegenerative diseases. It includes continuous activation of immune cells, microglia that leads to release and infiltration of inflammatory mediators such as nitrous oxide (NO), reactive oxygen species (ROS), cytokines, IL-6, IL-1β, and TNF-α into the brain that lead to degeneration of neurons, disrupting the BBB. Therefore, the microglial-mediated inflammation needs to be alleviated.[34] Drugs and natural products having anti-inflammatory properties have been estimated for their efficacy in neuroinflammation animal models.[35,36]

Treatment:

Neuroinflammation modulates the BDNF (Brain-derived neurotrophic factor) expressions, which decreases BDNF-mRNA levels. That leads to severe impairment of hippocampal function, memory, and motor decline with associated apoptosis in brain regions such as the cortex and hippocampus. In such a case, anti-inflammatory agents cannot fulfill the therapeutic purpose.[37] Therefore, researchers suggest that exogenous co-administration of BDNF with Simvastatin (Sim), a repurposed anti-inflammatory agent, will be beneficial in attenuating neuroinflammation.

Simvastatin, an inhibitor of the HMG-CoA reductase enzyme, is responsible for cholesterol biosynthesis in the body, which bestows to mediate neuroprotective action in the brain. It targets many pathways like inhibition of inflammatory mediators and microglial activation, attenuation of ions, stimulation of neurotrophic factor's expressions (BDNF, NGF), and suppression of apoptosis.

Since the drugs in the free form cannot reach CNS due to BBB, BCSFB, and efflux systems, it leads to decreased bioavailability, and consequently, the desired therapeutic response fails to accomplish. Accordingly, researchers developed a novel technique that includes intranasal co-delivery of PEG-PdLLA polymersome embedded with Sim and BDNF (Sim-BDNF-Ps) as illustrated in the fig.2. The combination markedly down-regulated brain levels of cytokines as compared to free drug and single drug-loaded polymersomes.[38] It effectively replenished the BDNF levels of the brain into two-fold that were depleted following neuroinflammation and additionally showed effective suppression of microglial activation.[34]

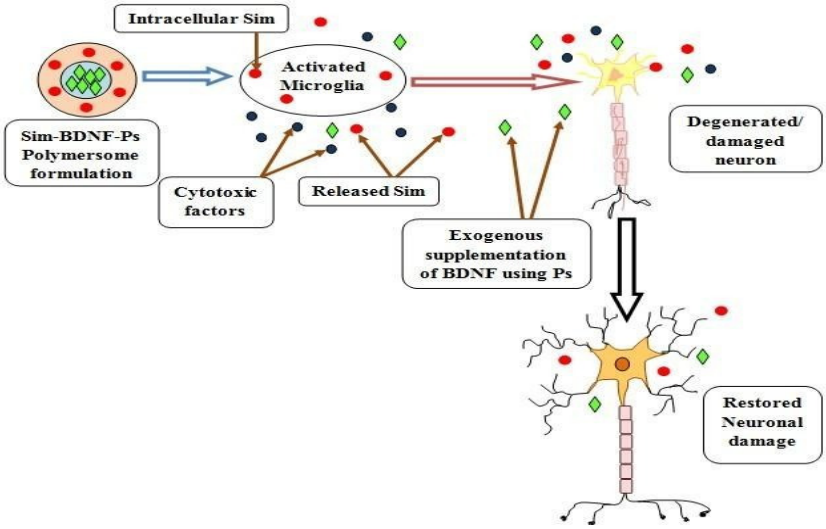

Fig.2: The intranasal co-delivery of PEG-PdLLA [poly(ethylene-glycol)-poly(D-Lactide)] polymersome embedded with Sim and BDNF (Sim-BDNF-Ps). Nose-to-brain co-delivery of repurposed simvastatin and BDNF synergistically replenish the BDNF levels in brain and attenuate LPS-induced neuroinflammation. Additionally, it showed effective suppression of microglial activation.

2. *Nanoformulation-based Peptide Delivery*

Pathogenesis:

Ischemic stroke occurs because of NMDAR (N-methyl-D-aspartate receptor)-mediated neurotoxicity, induced due to ischemia.[39] NR2B9C peptide can attenuate this neurotoxicity. But unfortunately, when administered alone, it can't cross BBB and neuronal membrane.[40]

Treatment:

Nano-formulation of wheat germ agglutinin (WGA) with NR2B9C peptide (WGA-NR2B9C-NPS) has been developed that can bypass BBB and reach the brain and neurons. WGA has a higher affinity towards WGA-receptor, present abundantly in the olfactory epithelium and neuronal surface, thus nano-formulation can be given intranasally to the brain.[41] Here, WGA acts as a carrier peptide, so it is also known as carrier-mediated transport. As it reaches the brain it binds to the receptor present on the neuronal membrane and undergoes receptor-mediated endocytosis. It forms an endosome (drug enclosed in nano-vesicle) which further ruptures to release the drug, thus blocks the calcium influx via NMDAR.[42] It inhibits the interaction of NMDAR with postsynaptic density protein-95 (PSD95) which inturn inhibits neuronal excitotoxicity. Thus prevent ischemic brain injury (experimented on rats) as described in fig.3. Certain experiments have proved that nanofiber-matrices or reservoirs are more advantageous over other nanoformulations or other conventional vehicles.[43] These drug-loaded nanofiber-formulations are suitable for a wide range of drugs due to high surface area and porosity, high drug payload, highly mucoadhesive, biodegradable, ease of administration that can be prepared under aseptic conditions. Another example is the olanzapine nanoparticulate drug delivery system that uses PLGA to provide brain targeting and sustained release. Olanzapine selectively binds to dopamine D2 and serotonin (5-HT2c) receptors to produce an antipsychotic effect.[44]

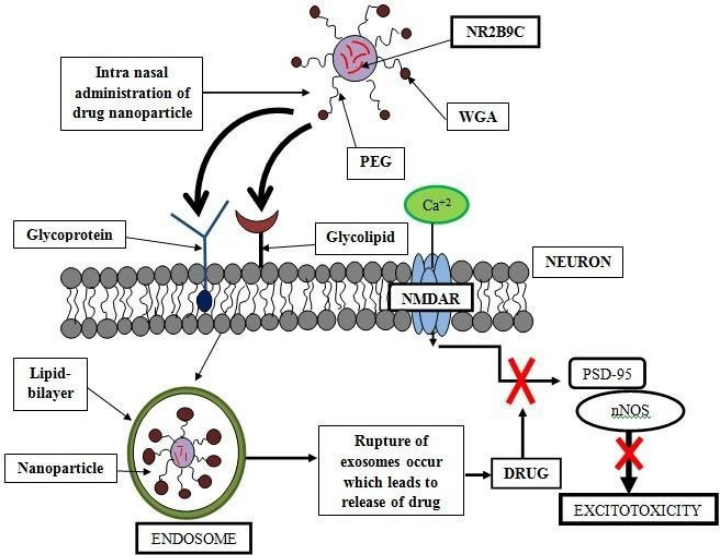

Fig. 3: Nanoformulation-based peptide delivery. It inhibits the interaction of NMDAR with postsynaptic density protein-95 (PSD-95) which inturn inhibits neuronal excitotoxicity thus prevent ischemic brain injury. NR2B9C – peptide, PEG – poly ethylene glycol, WGA – wheatgerm agglutinin, NMDAR – N-methyl-D-aspartate receptor.

3. *Cell-penetrating peptides (CPP)-based drug delivery:*

The nose-to-brain delivery has tremendous potential in treating various diseases likeAlzheimer's disease.

Pathogenesis and treatment:

Alzheimer's disease leads to cognitive impairment due to the extracellular deposition of beta-amyloid (Aβ) and intracellular accumulation of tau protein. Aβ is toxic to the neurons while the tau protein accumulation in the neuronal bodies displaces the cell organelles leading to cell dysfunction. Intranasal insulin administration shows good improvement in cognitive dysfunction. It minimizes the risk of the peptide hormone entering into the systemic circulation, thus hindering the blood insulin level. The nasal vasculature permits the entry of low molecular weight substances combating the main limitation of the IV route.[45] Insulin once administered intranasally, reaches the brain within 10 minutes and attains its peak point within 30 minutes. It then gets accumulated extracellularly and not axonally. In AD patients, there is decreased expression of the insulin receptor. The insulin signaling gets impeded due to the deposition of Aβ that competes with insulin for its binding to the insulin receptor and leads to numerous abnormalities like decreased PI3K expression, insulin receptor substrate expression, and AKT activation (decreases AKT phosphorylation). The impaired insulin signal transmission

activates GSK beta, a tau kinase enzyme responsible for tau hyperphosphorylation that ultimately leads to dementia or Alzheimer.[46] Hence, insulin is administered intranasally to increase the insulin level in the brain-CSF and prevent the binding of Aβ to insulin receptors, preventing the other side effects and improves cognition (memory) as shown in fig.4.

Several studies revealed that insulin aspart in solution form is better than insulin regular in solution or lipidized form.[47] As insulin regular forms hexamer, it primarily dissociates into monomers on reaching the brain receptors, acting upon the receptors. Whereas, insulin aspart doesn't form hexamer as it exists in a monomer form itself. Consequently, the patients receiving insulin aspart show faster remission rates (recovery in memory recall) as compared to the patients receiving insulin regular, where the dose and duration remain the same.

Macromolecular therapeutics such as proteins to treat CNS disorders are extremely difficult to administer due to their inability to cross BBB. The primary route for direct brain delivery is intracranial that is incredibly challenging and painful, yet it is being practiced. Noninvasive intranasal administration possesses an excellent advantage over other routes, as it circumvents the problem of crossing BBB.[48] As discussed earlier, it limits the entry of HMW substances due to the tight junctions present in the nasal mucosal membrane and restricts the axonal migration due to poor penetration capability. Hence, cell-penetrating peptide (CPP) is employed to deliver these macromolecules into the brain via intranasal administration. CPP can cross any barrier as it is cell-type independent and can transfer nanoparticles, genes, proteins, and macromolecules into the brain.

Intranasal co-administration of penetratin, a typical form of CPP, with supplemented insulin has shown tremendous improvement in mild cognitive impairment or in the early stage of dementia. Except for some severe cases, that shows a decrease in the potential of the therapy. Hence, an alternative for insulin such as exendin-4 (GLP-1 receptor agonist) is suggested to be administered. In severe cognitive dysfunction, insulin administration induces Aβ accumulation in the brain. It usually occurs due to the competitive degradation of insulin and Aβ by the insulin-degrading enzyme (IDE). Hence, this therapy is favorable in the early stage of dementia and is ineffective at severe stages of cognitive dysfunction.[49] Studies showed that spatial learning ability and insulin signaling improves when penetratin, insulin, and exendin-4 are co-administered intranasally for four weeks in severe cases. Also, some evidence suggests that response to intranasal insulin depends not only upon the dose but also upon the type of APOE gene the patient carries. IN insulin has been tested in several phase 2/3 clinical trial patients and has shown memory improvement. However, it is yet to be approved by US-FDA.

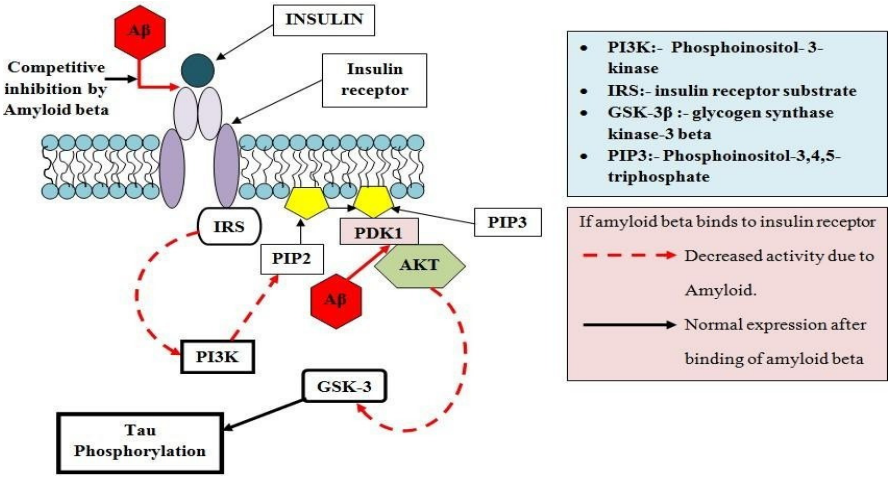

Fig. 4: Cell penetrating peptides-based drug delivery. Cell-penetrating (CPP) is employed to deliver insulin into the brain via intranasal administration to prevent the binding of Aβ to insulin receptors that improves cognition. CPP can cross any barrier as it is cell-type independent and can transfer nanoparticles, genes, proteins, and macromolecules into the brain.

Nasal Devices

There are different types of intranasal devices for the nose-to-brain delivery wherein the drug is targeted to the olfactory region and then to CNS. The selection of nasal devices depends upon the type of intranasal formulation. Nasal sprays and droppers are the most commonly used intranasal devices for lipid or liquid-based formulations.[50]

1. ***Liquid formulations*:** It dominates the intranasal market. Different types of formulations include:
   - **a)** Aqueous solutions
   - **b)** Emulsion
   - **c)** Suspension
   - **d)** Aerosols

These formulations get readily cleared due to gravitational force and mucociliary clearance. Thus, the drug hardly reaches the olfactory region impeding the dose delivered to the desired target. It leads to a decrease in the dose accuracy, restricting the exact dose from reaching the CNS. Newer devices have come up to reduce mucociliary clearance, overcoming the challenge. Benzalkonium chloride can be used to decrease ciliary movement and is also safe for long-term use. There is a risk of drugs entering into the systemic circulation via nasal vasculature.[50]Also side effects such as dry throat can be observed because the formulation being liquid gets drained into the pharynx.

Medical device: Nasal spray and nasal drops. But for dose accuracy, newer medical devices like the pressurized metered-dose inhalers (banned and replaced by nebulizer) are used but can lead to lung deposition due to Bi-directional™. To avoid this nebulizer was modified using a new technique such that it can create only sufficient pressure that is enough to close the palate, thus preventing the entry of drugs into the pharynx.[51] Additional devices used are mechanical nasal pump spray, liquid jets, metered dose spray pumps, single-dose sprays (Imitrex [sumatriptan] to treat migraine), multi-dose sprays, etc. For example, a Vianase atomizer is a device that is employed to deliver insulin to treat AD patients. Another example is VersiDoser®

2. ***Particulate formulations:***
   a) **Nanoparticle:** Nanoparticles have a lot of scopes as it protects the drug from biological or chemical degradation. It also aids the drug in escaping the reflux mechanism present in BBB due to drug encapsulation. Before releasing the drug at the desired site, the nanoparticle binds to the target receptor to initiate its action.[52]
   b) **Dry powders:** Powders can be more advantageous due to their better stability than liquid formulation. Usually, powders get moist when they enter the nasal mucosa furthermore are dissolved or get cleared off. Thus to overcome this, bioadhesives are used to decrease the ciliary movement and likewise the clearance.[51]

Medical device: Nasal sprays, breath-actuated inhalers, nasal insufflators, etc. For example, blister-based powder inhalers (prohaler™) from Aptar are employed to give apomorphine to treat Parkinson's disease. Bi-directional™ nasal devices are used for both liquid and powder as intranasal delivery. It overcomes the limitations of traditional nasal devices. However, it is in phase 3 clinical trials.[52] For example, a breath-powdered-Bi-directional™ device is used for the nose-to-brain delivery of midazolam that causes sedation.

3. ***Semisolids:***

Various mucoadhesives like carbopol, hypromellose, chitosan, etc., are used to increase the drug retention time and drug delivery. Various other excipients used are penetration enhancers, absorption enhancers, preservatives, etc. For the drug to reach the olfactory mucosa, it has to penetrate the mucous membrane. Accordingly, the excipients should possess a positive charge and hydrophobicity such that they can interact with the negatively charged mucin and can adhere accurately, evading the hydrophobic interactions. It leads to decreased clearance and can enhance drug penetration. Preservatives like benzalkonium chloride, chlorocresol, etc., are employed.[50]

Medical device: Nasal sprays, for example, metoclopramide HCl is used to treat nausea by interacting with dopamine receptors. It usually is in the solution form but gets converted into a gel as soon as it comes in contact with the nasal mucosa.

Advantages

1. A convenient, noninvasive, and painless route to deliver drugs directly to the brain via the nose, bypassing BBB, thus reducing the systemic side-effects and providing effective patient-friendly treatment.[3]
2. It offers more precise brain-targeted delivery of therapeutic agents that involves two neuronal pathways: olfactory and trigeminal nerve systems.
3. Due to ease of accessibility, self-medication is facilitated. Also, it is a needle-free drug application. Hence, no need for trained personnel. It ultimately improves patient compliance.[25]
4. Owing to the rich vasculature and high permeable structures consisting of a larger surface area, faster onset of action and enhancement in absorption are witnessed compared to the other routes. Hence, this route is suitable in case of emergency as an alternative to parenteral.
5. It decreases the frequency of the dose. It circumvents hepatic first-pass metabolism, providing increased drug concentration which in turn leads to a reliable bioavailability.
6. Enzymatic and chemical degradation of drugs in GIT is avoided. Therefore, it bestows a suitable route to deliver macromolecules such as peptides and proteins that get generally degraded in the harsh environment of GIT. They are not absorbed substantially into the systemic circulation following oral administration.
7. According to the clinical trials (Phase-I, II, and III), it has been reported that it is feasible to transport drugs via the intranasal route to the CNS. Hence, this route can deliver a wide range of medicines for the treatment of neurological disorders.[53-59]

Disadvantages

Although intranasal administration provides a better perspective than the other routes, it does possess certain limitations as follows:

1) Mucociliary clearance: It is the quick clearance of administered formulation from the nasal cavity. Generally, it occurs when the drug doesn't cross the nasal mucosa rapidly[23].
2) Enzymatic degradation: The epithelial barrier in the lumen of the nasal cavity consists of various peptidases enzymes that can cleave off the peptide or protein molecule in the nasal cavity itself. It fails to attain the specified therapeutic effect. But this barrier is often less considered as compared to low bioavailability.
3) Low bioavailability: As the molecular weight of the drug molecule increases, the efficacy of the delivery decreases. Only low molecular weight lipophilic substances can efficiently gain entry via this route. High molecular weight polar compounds like peptides and proteins have lower absorption rates, decreasing the bioavailability.
4) Mucosal damage: The excipients added in the formulation may irreversibly damage the cilia of the nasal mucosa and can lead to local side effects. Some chemical enhancers or surfactants are capable of dissolving the membrane at high concentrations. Continuous use of this route may lead to nasal infection or sometimes anosmia.
5) Pathological conditions: Allergic conditions such as a cold that cause nasal congestion may alter the rate of nasal clearance and considerably interfere with the therapeutic efficacy of the drug administered intranasally. Hence patients with severe cold and cough cannot be treated via this route.
6) Mechanical loss: There is a risk of drugs entering into other parts of the respiratory tract like the lungs that can alter the drug concentration reaching the target site. Hence, a proper device has to be employed to achieve targeted delivery.[53-59]

Nasal Grafting

Nasal grafting is the new and most promising drug delivery to the brain for enzyme replacement therapy and has potential advantages over intranasal drug delivery. It is an indirect route of the nose-to-brain drug delivery where the drug reaches the brain via a surgically grafted nasal mucosal membrane into the skull. It is a permanent grafting method that is irreversible. Besides the mucosal layer, a polypropylene reservoir is fixed into the skull with the help of cyano-acrylate adhesive. Bone screws and cement maintain the structural integrity of the skull. Such types of grafts are not only used as a route of drug delivery but also used to repair the skull deficits.[60]

Free mucosal grafts from the middle turbinate, inferior turbinate, and nasal septum are received as donor sites for the endo-nasal reconstruction of the skull base. that may be fixed adjacent to the sphenoid sinus. A large region of BCSFB or BBB is replaced with a permeable mucosa. The dose is applied topically to the grafted area for its delivery into the brain. It proves useful for the delivery of high molecular weight or polar drugs that enter the brain.[61] For example, treatment of Parkinson's disease:

Pathogenesis:

Parkinson's disease (PD) is a neurological disorder where the body's immune cells such as macrophages, damage dopaminergic neurons causing inflammation in the brain. It shows an accumulation of α-synuclein intracellularly.[62] The causes are unknown. PD patients have depression, anxiety, dementia, autonomic dysfunction, tremor, rigidity, bradykinesia, etc.

Treatment:

The treatment is done using the glial-derived neurotrophic factor (GDNF). GDNF is a protein that promotes the growth of dopaminergic neurons. It is applied topically on the permeable mucosal layer to be released from the reservoir into the brain via a transmucosal pathway.[63] Thus permeation of GDNF becomes easy. The effects obtained via the transmucosal pathway were similar to those observed with direct intraparenchymal injection. Therefore, the transmucosal pathway can be considered superior but clinical trials are not yet performed.[64]

Future Perspective

All over the world, the number of cases related to neurological disorders has been increased due to the rise in the aging population.[65] The current routes employed in the CNS treatment are difficult, painful, has delayed action, and are ineffective at times. Besides, BBB and BCSFB together make up an invincible obstacle to cross for a wide range of drugs.[39] Hence, the intranasal approach is emerging as a promising alternative route due to its various advantages along with overcoming the major challenge of bypassing the BBB. The underlying mechanism of the nose-to-brain delivery is still unknown, hence, it becomes essential to research the exact mechanism basically to know how the drug reaches specific areas of the brain through the nose for the treatment of neurological disorders.[66,67]

Despite possessing so many benefits, the intranasal route faces many challenges. Hence, various solutions like enzyme inhibitors or enzyme saturation are employed to overcome the challenge of enzyme degradation.

Bioadhesives/mucoadhesives and absorption/penetration enhancers used in the formulation circumvent mucociliary clearance increasing the retention time and improve the absorption, respectively. It enhances bioavailability.[68] Nanotechnology is being addressed efficiently as it provides promising solutions to these limitations. Several nanocarriers, for example, polymeric nanoparticles, solid lipid nanoparticles, liposomes, micelles, dendrimers, nanogels, nanoemulsions, and nanosuspensions have been studied to improve the BBB permeability.[69]

Stem cells have self renewing and multipotent activity and apart from this they possess incredible ability to pass through the BBB and reach the brain parenchyma following intranasal administration[70]. Researchers are increasing the adapting stem cell based nanotherapy for brain targeting via intranasal route in treating various neurological disorders which holds great promise as a therapeutic strategy[71].

According to recent studies, scientists have explored many drugs to treat AD and other neurological diseases.[72] Preclinical and clinical trials are performed to test the efficacies of drugs that turned out to be successful. Presently, not even a single drug is approved by US-FDA.[73] Thus, there is a dire need to focus on clinical trials to bring these drugs into the market. Hopefully, all the barriers might get resolved, and a wide range of drugs may come in the market shortly for disorders such as migraine, Alzheimer's disease, Parkinson's disease, stroke, MCI, etc.

References

1. Rubin LL, Staddon JM. The cell biology of the blood-brain barrier. Annu Rev Neurosci. 1999;22:11-28. Doi: 10.1146/annurev.neuro.22.1.11. PMID: 10202530.
2. Sharif Y, Jumah F, Coplan L, Krosser A, Sharif K, Tubbs RS. Blood brain barrier: A review of its anatomy and physiology in health and disease. Clin Anat. 2018 Sep;31(6):812-823. Doi: 10.1002/ca.23083. Epub 2018 Oct 18. PMID: 29637627.
3. Mittal D, Ali A, Md S, Baboota S, Sahni JK, Ali J. Insights into direct nose to brain delivery: current status and future perspective. Drug Deliv. 2014 Mar;21(2):75-86. Doi: 10.3109/10717544.2013.838713. Epub 2013 Oct 9. PMID: 24102636.
4. Fenstermacher J, Gross P, Sposito N, Acuff V, Pettersen S, Gruber K. Structural and functional variations in capillary systems within the brain. Ann N Y Acad Sci. 1988;529:21-30. Doi: 10.1111/j.1749-6632.1988.tb51416.x. PMID: 3395069.
5. Redzic Z. Molecular biology of the blood-brain and the blood-cerebrospinal fluid barriers: similarities and differences. Fluids Barriers CNS. 2011 Jan 18;8(1):3. Doi: 10.1186/2045-8118-8-3. PMID: 21349151; PMCID: PMC3045361.
6. Jones HC, Terasaki T. Fluids and Barriers of the CNS: a new journal encompassing Cerebrospinal Fluid Research. Fluids Barriers CNS. 2011 Jan 18;8(1):1. Doi: 10.1186/2045-8118-8-1. PMID: 21349146; PMCID: PMC3039835.
7. Sá-Pereira I, Brites D, Brito MA. Neurovascular unit: a focus on pericytes. Mol Neurobiol. 2012 Apr;45(2):327-47. Doi: 10.1007/s12035-012-8244-2. Epub 2012 Feb 28. PMID: 22371274.
8. Abbott NJ, Rönnbäck L, Hansson E. Astrocyte-endothelial interactions at the blood-brain barrier. Nat Rev Neurosci. 2006 Jan;7(1):41-53. Doi: 10.1038/nrn1824. PMID: 16371949.
9. Cohen Z, Molinatti G, Hamel E. Astroglial and vascular interactions of noradrenaline terminals in the rat cerebral cortex. J Cereb Blood Flow Metab. 1997 Aug;17(8):894-904. Doi: 10.1097/00004647-199708000-00008. PMID: 9290587.
10. Pardridge WM. Blood-brain barrier delivery. Drug Discov Today. 2007 Jan;12(1-2):54-61. Doi: 10.1016/j.drudis.2006.10.013. Epub 2006 Nov 13. PMID: 17198973.
11. Pardridge WM. Drug transport across the blood-brain barrier. J Cereb Blood Flow Metab. 2012 Nov;32(11):1959-72. Doi: 10.1038/jcbfm.2012.126. Epub 2012 Aug 29. PMID: 22929442; PMCID: PMC3494002.
12. Ghose AK, Herbertz T, Hudkins RL, Dorsey BD, Mallamo JP. Knowledge-Based, Central Nervous System (CNS) Lead Selection and Lead Optimization for CNS Drug Discovery. ACS Chem Neurosci. 2012 Jan 18;3(1):50-68. Doi: 10.1021/cn200100h. Epub 2011 Nov 2. PMID: 22267984; PMCID: PMC3260741.
13. Löscher W, Potschka H. Drug resistance in brain diseases and the role of drug efflux transporters. Nat Rev Neurosci. 2005 Aug;6(8):591-602. Doi: 10.1038/nrn1728. PMID: 16025095.
14. Bellettato CM, Scarpa M. Possible strategies to cross the blood-brain barrier. Ital J Pediatr. 2018 Nov 16;44(Suppl 2):131. Doi: 10.1186/s13052-018-0563-0. PMID: 30442184; PMCID: PMC6238258.
15. Gabathuler R. Approaches to transport therapeutic drugs across the blood-brain barrier to treat brain diseases. Neurobiol Dis. 2010 Jan;37(1):48-57. Doi: 10.1016/j.nbd.2009.07.028. Epub 2009 Aug 5. PMID: 19664710.

16. Wang Z, Xiong G, Tsang WC, Schätzlein AG, Uchegbu IF. Nose-to-Brain Delivery. J Pharmacol Exp Ther. 2019 Sep;370(3):593-601. Doi: 10.1124/jpet.119.258152. Epub 2019 May 24. PMID: 31126978.
17. Lochhead JJ, Thorne RG. Intranasal delivery of biologics to the central nervous system. Adv Drug Deliv Rev. 2012 May 15;64(7):614-28. Doi: 10.1016/j.addr.2011.11.002. Epub 2011 Nov 15. PMID: 22119441.
18. Dhuria SV, Hanson LR, Frey WH 2nd. Intranasal delivery to the central nervous system: mechanisms and experimental considerations. J Pharm Sci. 2010 Apr;99(4):1654-73. Doi: 10.1002/jps.21924. PMID: 19877171.
19. Selvaraj K, Gowthamarajan K, Karri VVSR. Nose to brain transport pathways an overview: potential of nanostructured lipid carriers in nose to brain targeting. Artif Cells Nanomed Biotechnol. 2018 Dec;46(8):2088-2095. Doi: 10.1080/21691401.2017.1420073. Epub 2017 Dec 28. PMID: 29282995.
20. Chen XQ, Fawcett JR, Rahman YE, Ala TA, Frey II WH. Delivery of Nerve Growth Factor to the Brain via the Olfactory Pathway. J Alzheimers Dis. 1998 Mar;1(1):35-44. Doi: 10.3233/jad-1998-1102. PMID: 12214010.
21. Dhuria SV, Hanson LR, Frey WH 2nd. Novel vasoconstrictor formulation to enhance intranasal targeting of neuropeptide therapeutics to the central nervous system. J Pharmacol Exp Ther. 2009 Jan;328(1):312-20. Doi: 10.1124/jpet.108.145565. Epub 2008 Oct 22. PMID: 18945930.
22. Crowe TP, Greenlee MHW, Kanthasamy AG, Hsu WH. Mechanism of intranasal drug delivery directly to the brain. Life Sci. 2018 Feb 15;195:44-52. Doi: 10.1016/j.lfs.2017.12.025. Epub 2017 Dec 22. PMID: 29277310.
23. Kashyap K, Shukla R. Drug Delivery and Targeting to the Brain Through Nasal Route: Mechanisms, Applications and Challenges. Curr Drug Deliv. 2019;16(10):887-901. Doi: 10.2174/1567201816666191029122740. PMID: 31660815.
24. Talegaonkar S, Mishra PR. Intranasal delivery: An approach to bypass the blood brain barrier. Indian J Pharmacol. 2004 May 5;36(3):140-147.
25. Putheti RR, Patil MC, Obire O. Nasal drug delivery in pharmaceutical and biotechnology: present and future. e-Journal of Science and Technology. 2009;4:1-21.
26. Schipper NG, Verhoef JC, Merkus FW. The nasal mucociliary clearance: relevance to nasal drug delivery. Pharm Res. 1991 Jul;8(7):807-14. Doi: 10.1023/a:1015830907632. PMID: 1924131.
27. Barile L, Vassalli G. Exosomes: Therapy delivery tools and biomarkers of diseases. Pharmacol Ther. 2017 Jun;174:63-78. Doi: 10.1016/j.pharmthera.2017.02.020. Epub 2017 Feb 12. PMID: 28202367.
28. Malm T, Loppi S, Kanninen KM. Exosomes in Alzheimer's disease. Neurochem Int. 2016 Jul;97:193-9. Doi: 10.1016/j.neuint.2016.04.011. Epub 2016 Apr 27. PMID: 27131734.
29. Yang T, Martin P, Fogarty B, Brown A, Schurman K, Phipps R, Yin VP, Lockman P, Bai S. Exosome delivered anticancer drugs across the blood-brain barrier for brain cancer therapy in Danio rerio. Pharm Res. 2015 Jun;32(6):2003-14. Doi: 10.1007/s11095-014-1593-y. Epub 2015 Jan 22. PMID: 25609010; PMCID: PMC4520542.
30. Lakhal S, Wood MJ. Exosome nanotechnology: an emerging paradigm shift in drug delivery: exploitation of exosome nanovesicles for systemic in vivo delivery of RNAi heralds new horizons for drug delivery across biological barriers. Bioessays. 2011 Oct;33(10):737-41. Doi: 10.1002/bies.201100076. PMID: 21932222.
31. Hornung S, Dutta S, Bitan G. CNS-Derived Blood Exosomes as a Promising Source of Biomarkers: Opportunities and Challenges. Front Mol Neurosci. 2020 Mar 19;13:38. Doi: 10.3389/fnmol.2020.00038. PMID: 32265650; PMCID: PMC7096580.
32. Upadhya R, Zingg W, Shetty S, Shetty AK. Astrocyte-derived extracellular vesicles: Neuroreparative properties and role in the pathogenesis of neurodegenerative disorders. J Control Release. 2020 Jul 10;323:225-239. Doi: 10.1016/j.jconrel.2020.04.017. Epub 2020 Apr 11. PMID: 32289328; PMCID: PMC7299747.
33. Jiang L, Dong H, Cao H, Ji X, Luan S, Liu J. Exosomes in Pathogenesis, Diagnosis, and Treatment of Alzheimer's Disease. Med Sci Monit. 2019 May 6;25:3329-3335. Doi: 10.12659/MSM.914027. PMID: 31056537; PMCID: PMC6515980.
34. Manickavasagam D, Lin L, Oyewumi MO. Nose-to-brain co-delivery of repurposed simvastatin and BDNF synergistically attenuates LPS-induced neuroinflammation. Nanomedicine. 2020 Jan;23:102107. Doi: 10.1016/j.nano.2019.102107. Epub 2019 Oct 23. PMID: 31655202.
35. Catorce MN, Gevorkian G. LPS-induced Murine Neuroinflammation Model: Main Features and Suitability for Pre-clinical Assessment of Nutraceuticals. Curr Neuropharmacol. 2016;14(2):155-64. Doi: 10.2174/1570159x14666151204122017. PMID: 26639457; PMCID: PMC4825946.

36. Ballatore C, Lee VM, Trojanowski JQ. Tau-mediated neurodegeneration in Alzheimer's disease and related disorders. Nat Rev Neurosci. 2007 Sep;8(9):663-72. Doi: 10.1038/nrn2194. PMID: 17684513.
37. Thorne RG, Frey WH 2nd. Delivery of neurotrophic factors to the central nervous system: pharmacokinetic considerations. Clin Pharmacokinet. 2001;40(12):907-46. Doi: 10.2165/00003088-200140120-00003. PMID: 11735609.
38. Alcalá-Barraza SR, Lee MS, Hanson LR, McDonald AA, Frey WH 2nd, McLoon LK. Intranasal delivery of neurotrophic factors BDNF, CNTF, EPO, and NT-4 to the CNS. J Drug Target. 2010 Apr;18(3):179-90. Doi: 10.3109/10611860903318134. PMID: 19807216; PMCID: PMC3732751.
39. Ying W. The nose may help the brain: intranasal drug delivery for treating neurological diseases. Future neurology. 2007 Dec 13;3(1):1-4. Doi: 10.2217/14796708.3.1.1
40. Li R, Huang Y, Chen L, Zhou H, Zhang M, Chang L, Shen H, Zhou M, Su P, Zhu D. Targeted delivery of intranasally administered nanoparticles-mediated neuroprotective peptide NR2B9c to brain and neuron for treatment of ischemic stroke. Nanomedicine. 2019 Jun;18:380-390. Doi: 10.1016/j.nano.2018.10.013. Epub 2018 Nov 11. PMID: 30428334.
41. Liu XF, Fawcett JR, Hanson LR, Frey WH 2nd. The window of opportunity for treatment of focal cerebral ischemic damage with noninvasive intranasal insulin-like growth factor-I in rats. J Stroke Cerebrovasc Dis. 2004 Jan-Feb;13(1):16-23. Doi: 10.1016/j.jstrokecerebrovasdis.2004.01.005. PMID: 17903945.
42. AD, Goyal AK, Rath G. Nanofibre in transmucosal drug delivery. Journal of drug delivery science and technology. 2018;43:379-387. doi: 10.1016/j.jddst.2017.11.008
43. Sofi HS, Abdal-Hay A, Ivanovski S, Zhang YS, Sheikh FA. Electrospun nanofibers for the delivery of active drugs through nasal, oral and vaginal mucosa: Current status and future perspectives. Mater Sci Eng C Mater Biol Appl. 2020 Jun;111:110756. Doi: 10.1016/j.msec.2020.110756. Epub 2020 Feb 19. PMID: 32279775.
44. Seju U, Kumar A, Sawant KK. Development and evaluation of olanzapine-loaded PLGA nanoparticles for nose-to-brain delivery: in vitro and in vivo studies. Acta Biomater. 2011 Dec;7(12):4169-76. Doi: 10.1016/j.actbio.2011.07.025. Epub 2011 Jul 30. PMID: 21839863.
45. Kamei N, Okada N, Ikeda T, Choi H, Fujiwara Y, Okumura H, Takeda-Morishita M. Effective nose-to-brain delivery of exendin-4 via coadministration with cell-penetrating peptides for improving progressive cognitive dysfunction. Sci Rep. 2018 Dec 5;8(1):17641. Doi: 10.1038/s41598-018-36210-9. PMID: 30518944; PMCID: PMC6281676.
46. Morris JK, Burns JM. Insulin: an emerging treatment for Alzheimer's disease dementia? Curr Neurol Neurosci Rep. 2012 Oct;12(5):520-7. Doi: 10.1007/s11910-012-0297-0. PMID: 22791280; PMCID: PMC3540744.
47. Medeiros R, Baglietto-Vargas D, LaFerla FM. The role of tau in Alzheimer's disease and related disorders. CNS Neurosci Ther. 2011 Oct;17(5):514-24. Doi: 10.1111/j.1755-5949.2010.00177.x. Epub 2010 Jun 14. PMID: 20553310; PMCID: PMC4072215.
48. Chatterjee S, Ambegaokar SS, Jackson GR, Mudher A. Insulin-Mediated Changes in Tau Hyperphosphorylation and Autophagy in a Drosophila Model of Tauopathy and Neuroblastoma Cells. Front Neurosci. 2019 Aug 2;13:801. Doi: 10.3389/fnins.2019.00801. PMID: 31427921; PMCID: PMC6688711.
49. Lin T, Liu E, He H, Shin MC, Moon C, Yang VC, Huang Y. Nose-to-brain delivery of macromolecules mediated by cell-penetrating peptides. Acta Pharm Sin B. 2016 Jul;6(4):352-8. Doi: 10.1016/j.apsb.2016.04.001. Epub 2016 May 26. PMID: 27471676; PMCID: PMC4951590.
50. Gänger S, Schindowski K. Tailoring Formulations for Intranasal Nose-to-Brain Delivery: A Review on Architecture, Physico-Chemical Characteristics and Mucociliary Clearance of the Nasal Olfactory Mucosa. Pharmaceutics. 2018 Aug 3;10(3):116. Doi: 10.3390/pharmaceutics10030116. PMID: 30081536; PMCID: PMC6161189.
51. Djupesland PG. Nasal drug delivery devices: characteristics and performance in a clinical perspective-a review. Drug Deliv Transl Res. 2013 Feb;3(1):42-62. Doi: 10.1007/s13346-012-0108-9. Epub 2012 Oct 18. PMID: 23316447; PMCID: PMC3539067.
52. Hong SS, Oh KT, Choi HG, Lim SJ. Liposomal Formulations for Nose-to-Brain Delivery: Recent Advances and Future Perspectives. Pharmaceutics. 2019 Oct 17;11(10):540. Doi: 10.3390/pharmaceutics11100540. PMID: 31627301; PMCID: PMC6835450.
53. Bourganis V, Kammona O, Alexopoulos A, Kiparissides C. Recent advances in carrier mediated nose-to-brain delivery of pharmaceutics. Eur J Pharm Biopharm. 2018 Jul;128:337-362. Doi: 10.1016/j.ejpb.2018.05.009. Epub 2018 May 4. PMID: 29733950.
54. Pires A, Fortuna A, Alves G, Falcão A. Intranasal drug delivery: how, why and what for? J Pharm Pharm Sci. 2009;12(3):288-311. Doi: 10.18433/j3nc79. PMID: 20067706.

55. Agrawal M, Saraf S, Saraf S, Antimisiaris SG, Chougule MB, Shoyele SA, Alexander A. Nose-to-brain drug delivery: An update on clinical challenges and progress towards approval of anti-Alzheimer drugs. J Control Release. 2018 Jul 10;281:139-177. Doi: 10.1016/j.jconrel.2018.05.011. Epub 2018 May 24. PMID: 29772289.
56. Alexander A, Saraf S. Nose-to-brain drug delivery approach: A key to easily accessing the brain for the treatment of Alzheimer's disease. Neural Regen Res. 2018 Dec;13(12):2102-2104. Doi: 10.4103/1673-5374.241458. PMID: 30323136; PMCID: PMC6199953.
57. Cox A, Vinciguerra D, Re F, Magro RD, Mura S, Masserini M, Couvreur P, Nicolas J. Protein-functionalized nanoparticles derived from end-functional polymers and polymer prodrugs for crossing the blood-brain barrier. Eur J Pharm Biopharm. 2019 Sep;142:70-82. Doi: 10.1016/j.ejpb.2019.06.004. Epub 2019 Jun 6. PMID: 31176723.
58. More SS, Vince R. Design, synthesis and biological evaluation of glutathione peptidomimetics as components of anti-Parkinson prodrugs. J Med Chem. 2008 Aug 14;51(15):4581-8. Doi: 10.1021/jm800239v. Epub 2008 Jul 24. PMID: 18651729.
59. Denora N, Laquintana V, Lopedota A, Serra M, Dazzi L, Biggio G, Pal D, Mitra AK, Latrofa A, Trapani G, Liso G. Novel L-Dopa and dopamine prodrugs containing a 2-phenyl-imidazopyridine moiety. Pharm Res. 2007 Jul;24(7):1309-24. Doi: 10.1007/s11095-007-9255-y. Epub 2007 Apr 3. PMID: 17404814.
60. Marianecci C, Rinaldi F, Hanieh PN, Di Marzio L, Paolino D, Carafa M. Drug delivery in overcoming the blood-brain barrier: role of nasal mucosal grafting. Drug Des Devel Ther. 2017 Jan 27;11:325-335. Doi: 10.2147/DDDT.S100075. PMID: 28184152; PMCID: PMC5291459.
61. Talegaonkar S, Mishra PR. Intranasal delivery: An approach to bypass the blood brain barrier. Indian J Pharmacol. 2004 May 5;36(3):140-147.
62. Beitz JM. Parkinson's disease: a review. Front Biosci (Schol Ed). 2014 Jan 1;6:65-74. Doi: 10.2741/s415. PMID: 24389262.
63. Zhao Y, Haney MJ, Gupta R, Bohnsack JP, He Z, Kabanov AV, Batrakova EV. GDNF-transfected macrophages produce potent neuroprotective effects in Parkinson's disease mouse model. PLoS One. 2014 Sep 17;9(9):e106867. Doi: 10.1371/journal.pone.0106867. PMID: 25229627; PMCID: PMC4167552.
64. Agrawal M, Saraf S, Saraf S, Antimisiaris SG, Chougule MB, Shoyele SA, Alexander A. Nose-to-brain drug delivery: An update on clinical challenges and progress towards approval of anti-Alzheimer drugs. J Control Release. 2018 Jul 10;281:139-177. Doi: 10.1016/j.jconrel.2018.05.011. Epub 2018 May 24. PMID: 29772289.
65. Musumeci T, Bonaccorso A, Puglisi G. Epilepsy Disease and Nose-to-Brain Delivery of Polymeric Nanoparticles: An Overview. Pharmaceutics. 2019 Mar 13;11(3):118. Doi: 10.3390/pharmaceutics11030118. PMID: 30871237; PMCID: PMC6471219.
66. Wong HL, Wu XY, Bendayan R. Nanotechnological advances for the delivery of CNS therapeutics. Adv Drug Deliv Rev. 2012 May 15;64(7):686-700. Doi: 10.1016/j.addr.2011.10.007. Epub 2011 Nov 7. PMID: 22100125.
67. Jadhav KR, Gambhire MN, Shaikh IM, Kadam VJ, Pisal SS. Nasal drug delivery systems-factors affecting and applications. Current drug therapy. 2012Mar01;2(1):27-38. Doi: 10.2174/157488507779422374
68. Mistry A, Stolnik S, Illum L. Nanoparticles for direct nose-to-brain delivery of drugs. Int J Pharm. 2009 Sep 8;379(1):146-57. Doi: 10.1016/j.ijpharm.2009.06.019. Epub 2009 Jun 23. PMID: 19555750.
69. Brasnjevic I, Steinbusch HW, Schmitz C, Martinez-Martinez P; European NanoBioPharmaceutics Research Initiative. Delivery of peptide and protein drugs over the blood-brain barrier. Prog Neurobiol. 2009 Apr;87(4):212-51. Doi: 10.1016/j.pneurobio.2008.12.002. Epub 2009 Jan 24. PMID: 19395337.
70. Dali P, Shende P. Advances in Stem Cell Therapy for Brain Diseases via the Intranasal Route. Curr Pharm Biotechnol. 2021;22(11):1466-1481. doi: 10.2174/1389201021666201218130947. PMID: 33342406.
71. Zhang YT, He KJ, Zhang JB, Ma QH, Wang F, Liu CF. Advances in intranasal application of stem cells in the treatment of central nervous system diseases. Stem Cell Res Ther. 2021 Mar 24;12(1):210. doi: 10.1186/s13287-021-02274-0. PMID: 33762014; PMCID: PMC7992869.
72. Pavan B, Dalpiaz A, Ciliberti N, Biondi C, Manfredini S, Vertuani S. Progress in drug delivery to the central nervous system by the prodrug approach. Molecules. 2008 May 1;13(5):1035-65. Doi: 10.3390/molecules13051035. PMID: 18560328; PMCID: PMC6245073.
73. Schaefer ML, Böttger B, Silver WL, Finger TE. Trigeminal collaterals in the nasal epithelium and olfactory bulb: a potential route for direct modulation of olfactory information by trigeminal stimuli. J Comp Neurol. 2002 Mar 12;444(3):221-6. Doi: 10.1002/cne.10143. Erratum in: J Comp Neurol 2002 Jul 8;448(4):423. PMID: 11840476.