NANOTECHNOLOGY-MEDIATED NOSE TO BRAIN DRUG DELIVERY FOR PARKINSON’S DISEASE: A MINI REVIEW

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ABSTRACT
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. Nanoparticle (NP) therapeutics is an emerging modality for the treatment of Parkinson’s disease (PD) as it offers targeted delivery and enhances the therapeutic efficacy and/or bioavailability of neurotherapeutics. This is relevant in the field of drug delivery as well as for new developments in nanotechnology.


INTRODUCTION
Diseases of the Central Nervous System (CNS) such as schizophrenia, meningitis, migraine, Parkinson’s disease and Alzheimer’s disease require delivery of the drug to the brain for treatment. However such transport remains problematic, especially for hydrophilic drugs and large molecular weight drugs, due to the impervious nature of the endothelial membrane separating the systemic circulation and central interstitial fluid, the Blood–Brain Barrier (BBB). Since long time, delivery of neurotherapeutics to the brain has been more challenging for researchers, since this organ benefits from a very efficient, highly dense membrane system recognized as blood–brain barrier (BBB). BBB together with enzymes restricts the
entry of substances and many neurotherapeutics for maintaining internal milieu of the brain.\textsuperscript{[1, 2, 3, 4]}

PD is a progressive neurodegenerative disorder affecting 1–2\% of population over the age of 65. It occurs primarily due to death of dopaminergic neurons of nigrostriatal and mesolimbic system, causing movement abnormalities, besides cognitive and psychiatric manifestations. Many cellular mechanisms are believed to be responsible for neuronal death in PD, such as endoplasmic reticulum stress, proteasomal and mitochondrial dysfunction.\textsuperscript{[5, 6]}

Intranasal (i.n.) administration offers lots of benefits in the treatment of neurodegenerative disorders (NDs) through BBB bypass drug delivery. A number of advantages of intranasal administration are particularly attractive such as non-invasive, rapid systemic absorption, fast onset of action, avoidance of first-pass metabolism, increasing drug bioavailability, and less systemic side effects\textsuperscript{[7]}

It is the purpose of this review to pinpoint the potential of nanotechnology-mediated drug delivery through a transport route from the nose to the brain, particularly in the treatment of PD. To the best of our information, there is not a single literature available till date discussing the potential of nanotechnology-mediated drug delivery for PD via nose to brain transport route and our efforts are prime venture in this direction.

**Advantages of intranasal drug delivery**

- Rapid drug absorption via highly vascularized mucosa
- Ease of administration, non-invasive
- Improved bioavailability
- Improved convenience and compliance
- Self-administration
- Large nasal mucosal surface area for dose absorption
- Avoidance of the gastrointestinal tract and first-pass metabolism
- Rapid onset of action
- Lower side effects
- Drugs which cannot be absorbed orally may be delivered to the Systemic circulation through nasal drug delivery system.
- Convenient route when compared with parenteral route for long term therapy.
Bioavailability of larger drug molecules can be improved by means of absorption enhancer or other approach.

Disadvantages of intranasal drug delivery
- Some drugs may cause irritation to the nasal mucosa
- Nasal congestion due to cold or allergies may interfere with absorption of drug.
- Drug delivery is expected to decrease with increasing molecular weight.
- Frequent use of this route leads to mucosal damage
- The amount of drug reaches to different regions of the brain and spinal cord varies with each agent.

Parkinson’s disease
Parkinsonism disease is a leading cause of neurological disability; it is the second most common progressive neurodegenerative disorder. The effect of the parkinsonism disease reaches 1-2% in people over the age of 50. It has no gender preference and has a worldwide distribution. The symptoms of parkinsonism disease are largely related to progressive loss of dopamine in the basal ganglia. The main cause of the Parkinson's disease is unknown, but it is well characterized. The degeneration of brain cell occurs primarily in the midbrain region area called as substantia nigra. Normally, substantia nigra brain cells communicate with another region of the brain called as the striatum via chemical messenger called dopamine also loss of cell in the substantia nigra results decrease in the levels of available dopamine. The exogenous substitution with dopamine agonists on the dopamine prodrug, levodopa corrects the mechanical disorders at early stage of parkinsonism disease. The levodopa is converted into dopamine after its administration and stored in the dopaminergic neurons, still levodopa is considered as best drug in the treatment of parkinsonism disease.

Drugs for Parkinsonism Disease: - Parkinson's disease is a chronic disorder that requires broad-based management including patient and family education, support group services, general wellness maintenance, physiotherapy, exercise. There are number of drugs which are used in the treatment of Parkinson’s disease. These contains agonists of dopamine (e.g. apomorphine), cholinesterase inhibitors (e.g. donepezil) etc and provide safety, compliance, feasibility and short term/long term efficacy to the patients.\[8\]
Fig 1. Treatment options for Parkinson’s disease and the mechanisms of action.

Nasal anatomy and physiology

Anatomy and Physiology

In humans the functions of the nasal cavity are breathing and olfaction. It also affords an important protective activity once it filters, heat and humidify the inhaled air before reaching the lowest airways. Nasal cavity is lined with mucus layer and hairs which are involved in those functions are trapping inhaled particles and pathogens. Moreover, mucociliary clearance, immunological activities and metabolism of endogenous substances are also essential functions of nasal structures. The nasal cavity is a space situated above the oral cavity and hard palate and below the skull base and intracranial compartment. The nasal septum consists of cartilage in its front end and bone towards the back of the nose. The perpendicular plate of the ethmoid bone, vomer bone, and maxilla bone these three gives nasal septum. The nasal septum is sometimes crooked or off-midline, which leads to narrowing of one or both sides of the nasal cavity. The left and right nasal cavities become continuous in the back of the nose via the opening to the nasopharynx are called as the choana. In this area, the nasal cavity transitions into the nasopharynx. The nasopharynx contains a collection of centrally located lymphoid tissue called the adenoids. The human nasal cavity has a total volume of 15-20mL and a total surface area of 150 cm$^2$. It is divided by middle septum into two symmetrical halves, each one opening at the face through nostrils and extending posterior to the nasopharynx. Both symmetrical halves consist of four areas
(nasal vestibule, atrium, respiratory region and olfactory region) that are distinguished according to their anatomic and histological characteristics.

**Respiratory Region:** The respiratory epithelium is made of with four types of cells are non-ciliated and ciliated columnar cells, basal cells and goblet cells. These cells facilitate active transport processes such as the exchange of water and ions between cells and motility of cilia and also to prevent drying of the mucosa by trapping moisture in order to facilitate mucociliary clearance. A viscous gel layer, the mucus blanket floats on the serous fluid layer. The viscous gel layer is moved along by the hook shaped cilia termini during the energy dependent ‘effective stroke’ phase of the ciliary motion Cilia are up to 7mm in length when fully extended but can fold to half this length during the recovery stroke where the hook terminus detaches from the gel layer and moves immersed in the sol layer in the opposite direction to the gel layer movement The cilia beat with a frequency of 1000 strokes per min. Hence the mucus moves only in one direction from the anterior to the posterior part of the nasal cavity to the nasopharynx.

**Olfactory Region:** Smell allows humans and animals with olfactory receptors to identify food, mates, predators, and provides both sensual pleasure as well as warnings of danger. The olfactory region of the two nasal passages in humans is a area of about 2.5 square centimeters containing in total of about 50 million primary sensory receptor cells. The olfactory region consists of cilia projecting down out of the olfactory epithelium into a layer of mucous which is about 60 microns thick. This mucous layer is a lipid-rich secretion that bathes the surface of the receptors at the epithelium surface. The mucous layer is produced by the Bowman’s glands which reside in the olfactory epithelium. The mucous lipids assist in transporting the odorant molecules as only volatile materials that are soluble in the mucous can interact with the olfactory receptors and produce the signals that our brain interprets as odor.[10]
Mechanism of Drug Absorption from Nose

The initial step in the absorption of drug from the nasal cavity is passage through the mucus; large/charged particles may find it more difficult to cross. But small unchanged particles easily pass through this layer. The mechanisms for absorption through the nasal mucosa. These include paracellular transport via movement between cell and transcytosis by vesicle carrier’s transcellular or simple diffusion across the membrane.

1. The first mechanism includes aqueous route of transport, which is also called as the paracellular route. This is slow and passive route. Inverse log-log relationship between intranasal absorption and the molecular weight of water-soluble compounds. Poor bioavailability was observed for drugs with a molecular weight greater than 1000Daltons9.

2. The second mechanism is transport through a lipoidal route is known as transcellular process and is responsible for the transport of lipophilic drugs that show a rate dependency on their lipophilicity. Drugs also cross cell membranes by an active transport route via carrier-mediated means or transport through the opening of tight junctions. For example, Chitosan, a natural biopolymer opens tight junctions between epithelial cells to facilitate drug transport.11
BBB
The BBB consists of walls of capillaries that separate brain from circulating blood (Fig. 2). In the human brain there are approximately 100 billion capillaries that have a net surface area of nearly 20 square meters. Despite its enormous surface area, the BBB lacks intercellular cleft and fenestrae and significantly restricts the entry of solutes to the brain from the periphery. Its low permeability is attributed, in large part, to BMVEC (Bovine Brain Microvascular Endothelial Cells) which forms tight junctions and has low pinocytic activity some relatively lipophilic and low molecular weight substances can transport across the BMVEC by passive diffusion. However, a large number of lipophilic compounds are rapidly effluxed from the brain into the blood by extremely effective drug efflux systems expressed in the BBB. These efflux systems include P-glycoprotein (Pgp), multidrug resistance proteins (MRPs), breast cancer resistance protein (BCRP), and the multi-specific organic anion transporter (MOAT). There is also an enzymatic barrier to drug transport in the BMVEC. Activity of many enzymes that participate in the metabolism and inactivation of endogenous compounds, such as g-glutamyltranspeptidase, alkaline phosphatase, and aromatic acid decarboxylase is elevated in cerebral microvessels.

Fig. 2: The BBB is formed by BMVEC (see insert) that form tight junctions and express different transport systems such as Pgp, glucose transporter (GLUT1), large amino acid transporter (LAT1), excitatory amino acid transporters (EAAT1-3), transferrin receptor and others.
Barriers for nasal drug absorption

Enzymatic barrier
The nasal mucosa contains enzymes such as cytochrome P450-dependent monooxygenase, carboxyl esterase and amino peptidase. Nasal delivery avoids hepatic first-pass metabolism to some extent, the enzymes present in nasal mucosa provides a pseudo-first-pass effect.[21] The role of the enzymatic barrier is to protect the lower respiratory airways from toxic agents. In addition, there are various barriers in the nasal membrane for protection from the microorganisms, allergens and irritating substances from the environment that must be overcome by drugs before they can be absorbed into the systemic circulation.[22]

Mucociliary clearance
Particles entrapped in the mucus layer are transported and cleared from the nasal cavity. The combined action of the mucus layer and cilia is called mucociliary clearance.[23] This is an defence mechanism of the respiratory tract to protect against noxious inhaled materials. Mucus traps the particles of dust, bacteria and drug substances and is transported towards the nasopharynx at a speed of 5 - 8 mm/min where it is swallowed. The normal mucociliary transit time in humans has been reported to be 13 to 15 min.[24]

Protective barriers:
Small molecular weight and uncharged substances can easily pass through this layer. But larger or charged particles are difficult to cross. Mucin, the protein in the mucus, has the potential to bind to solutes, hindering diffusion. Additionally, structural changes in the mucus layer are possible as a result of environmental changes such as pH, temperature etc.[25] The nasal membrane is a physical barrier and the mucociliary clearance is a temporal barrier to drug absorption across the nasal epithelium.[26]

Nanotechnology
National Nanotechnology Initiative (http://www.nano.gov) defines nanotechnology as the production of materials in the scale between 1 and 100 billionths of a meter (1–100 nm), at least in one dimension. NP therapeutics is a budding treatment option for NDs as a consequence of targeted delivery and enhanced therapeutic efficacy and/or bioavailability of neurotherapeutics.[27] Interaction of nanotechnology with target sites induces physiological responses and minimizes side effects; thereby revolutionize the treatment of PD.[28, 29, 30] Nanoparticles may offer an improvement to nose-to-brain drug delivery since they are able to protect the encapsulated drug from biological and/or chemical degradation, and extracellular
transport by P-gp efflux proteins. This would increase CNS availability of the drug. A high relative surface area means that these vectors will release drug faster than larger equivalents; a property desirable where acute management of pain is required. Their small diameter potentially allows nanoparticles to be transported transcellularly through olfactory neurons to the brain via the various endocytic pathways of sustentacular or neuronal cells in the olfactory membrane, as described above. Surface modification of the nanoparticles could achieve targeted CNS delivery of a number of different drugs using the same ‘platform’ delivery system which has known and well characterized biophysical properties and mechanism(s) of transit into the CNS.\cite{31}

Nanosystems explored for advanced experimental treatment of Parkinson’s disease

Brain-targeted delivery of dopamine using nanosystems in PD

Various approaches of delivering dopamine (DA) to the brain with particular focus on the use of redox-based delivery systems for the targeted delivery and localized release of DA in the brain have been performed.\cite{73} Results have shown that DA can be successfully delivered into the brain, accompanied by localized release and metabolism, which allows the execution of appropriate pharmacological responses. These results open the possibility of treating a variety of NDs, since normally the BBB restricts the entry of polar compounds such as DA into the brain parenchyma.

Desirable properties of nanoparticles for brain targeting after intranasal administration

In conjunction with these fundamental properties, NPs must possess the below mentioned properties, in general, for targeting the brain.

(i) Non-toxic, biocompatible and biodegradable.
(ii) Physical stability, in vitro as well as in vivo.
(iii) Avoidance from reticuloendothelial system (RES) which may prolong the blood circulation time.
(iv) Scalable and cost effective manufacturing process.
(v) Amenable to small molecules, proteins and peptides or nucleic acids.
(vi) Formulation stability, minimal nanoparticle excipient-induced drug alteration (chemical degradation/alteration, protein denaturation); and (vii) Controlled drug release profiles.

The nanoparticulate carriers are thought to elicit its action through one of the following principal mechanisms.
(i) Enhanced retention of bioadhesive nanoparticle formulation for prolonged period of time on the mucosal surface of nasal cavity that would enhance the delivery of neurotherapeutics across the endothelial cell layer and thereby to the brain regions.

(ii) Ability of NPs of transiently opening of the tight junctions of the mucosal epithelium. The neurotherapeutic could then permeate through the tight junctions either in free form or in bound form together with the NPs.

(iii) Endocytosis or transcytosis of the NPs by the endothelial cells followed by the release of the drug within these cells and its delivery to the brain.

**Pathways and mechanisms for nose to brain delivery of nanomaterial’s**

Previously, our group had extensively reviewed the pathways and mechanisms for direct nose to brain delivery of neurotherapeutics. While the precise and core mechanisms underlying intranasal drug delivery to the brain are not entirely investigated so far, a cluster of evidences demonstrated that pathways involving nerves (olfactory and trigeminal) connecting the nasal passages to the brain and spinal cord are very crucial. In addition, pathways involving the cerebrospinal fluid and lymphatic system have been employed in the transport of therapeutic modalities from the nasal cavity to the brain. It is plausible that, a combination of these pathways is responsible, although one pathway may predominate, depending on the properties of neurotherapeutics, characteristics of formulation and the drug delivery device used [32]

**Nanotechnology for PD**

Nano-enabled approaches have been reported for the delivery of various neurotherapeutics intended for the therapy of PD. Nano medicine can be defined as “the design and manipulation of nanoparticles, particularly as applied to the medical diagnosis and treatment of disease.” Nanotechnology is being applied ingeniously to provide new, patient-friendly solutions to delivering drugs. Delivering a drug correctly, to the right part of the body, causes numerous challenges.

**Formulation Strategies**

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.
**Chitosan nanoparticles**

Currently, NPs prepared from cationic polysaccharide chitosan (CS) have shown promising results in nose to brain drug delivery because of its excellent intrinsic properties like low toxicity, excellent biocompatibility, high loading and entrapment efficiency and ability of delivering hydrophilic molecules.

Bromocriptine-loaded chitosan nanoparticles (BRC-CS NPs) by an ionic gelation with tripolyphosphate (TPP; 0.175% w/v) as anion. The chitosan used was of medium Mw and with degree of deacetylation of about 85%, prepared in acetic acid (2% v/v; pH 3.5). The concentration of chitosan used was 0.175% w/v. Biodistribution and pharmacokinetic studies revealed the higher brain/blood ratios of intranasal BRC-CS NPs compared to intranasal BRC solution and intravenous BRC-CS NPs, indicating the direct nose to brain transport of BRC along olfactory or trigeminal nerve pathways bypassing the BBB. This was further confirmed by higher drug targeting index (DTI), drug targeting efficiency (DTE) and drug transport percentage (DTP).[33]

The enhanced direct nose to brain drug delivery effect of chitosan formulations is suggested to be attributable to a combination of:

1) The passive targeting ability of chitosan by mucoadhesion resulting in increased residence time of formulation over the olfactory region and
2) The increased permeability of nasal epithelia to drug due to tight junction opening between apical cells.

**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 (davg:85-90nm) 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.\cite{34,35}

**Solid lipid nanoparticles**

Solid lipid nanoparticles (SLN), the first generation of lipid NPs, have barely studied for i.n. delivery of anti-Parkinsonian agents. Preparation of surface-modified SLN for the i.n. delivery of ropinirole hydrochloride has been reported. The surface modification of fabricated SLN using cationic stearylamine-induced cat-ionic charge on the surface of SLN, and also contributed to the enhanced entrapment efficiency and sustained drug release. The nasal toxicity studies revealed the non-irritant nature of the SLN formulation as studied on the sheep nasal mucosa. SEM analysis showed nearly spherical shape of SLN with a smooth surface, which is desirable for the efficient deposition of SLN in the nasal cavity. The SLN formulation aids in reducing the dose and dosing frequency, thereby maximizing the therapeutic index of the selected drug candidate.\cite{36}

**Nanoemulsions and mucoadhesive nanoemulsions**

Nanoemulsions (NEs), by virtue of their lipophilic nature and low globule size, are widely explored as a delivery system to enhance uptake across the nasal mucosa. The addition of mucoadhesive agents such as polyelectrolyte polymers help in the retention of formulation on the nasal mucosa, thereby providing an extended delivery of the drug to the olfactory region and henceforth to the brain. The NEs modified with mucoadhesive agents are referred to as mucoadhesive nanoemulsions (MNEs).

The formulation and development of ropinirole-loaded NE and MNE for i.n. administration as a better management option for the treatment of PD. The formulation was characterized, primarily, by physico-chemical investigations like particle size (58.61 ± 5.18), Polydispersity index (0.201), viscosity (31.42 ± 6.97), stability, dilution capability and ability to improve i.n. flux. The ex-vivostudies showed a significant higher drug translocation in different parts of the Wistar rat brain. From the in vitro, ex-vivo and invivo evaluation, the authors have concluded that the NEs and MNEs could be a promising approach for the IN delivery of ropinirole hydrochloride and utilized as a better treatment option for PD.\cite{37}
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CS NPs: Chitosan nanoparticles; PEG: Poly(ethylene glycol); HCl: Hydrochloride; SLN: solid lipid nanoparticles; NE: nanoemulsion; MNE: mucoadhesivenanoemulsion;

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.

REFERENCES

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