Current Neuropharmacology, 2023, *21*, 493-516

REVIEW ARTICLE

Nanotechnological Advances for Nose to Brain Delivery of Therapeutics to Improve the Parkinson Therapy

Dharmendra K. Khatri[1,*], Kumari Preeti[1,#], Shivraj Tonape[1,#], Sheoshree Bhattacharjee[1], Monica Patel[1], Saurabh Shah[2], Pankaj K. Singh[2], Saurabh Srivastava[2], Dalapathi Gugulothu[3], Lalitkumar Vora[4] and Shashi B. Singh[1,*]

[1]*Department of Pharmacology & Toxicology, National Institute of Pharmaceutical Education and Research, Hyderabad, Telangana State, India;* [2]*Department of Pharmaceutics, National Institute of Pharmaceutical Education and Research, Hyderabad, Telangana State, India;* [3]*Department of Pharmaceutics, Delhi Pharmaceutical Sciences and Research University (DPSRU), New Delhi-110017, India;* [4]*School of Pharmacy, Queen's University Belfast, 97 Lisburn Road, Belfast-BT9 7BL, UK*

ARTICLE HISTORY

Received: January 25, 2022
Revised: February 06, 2022
Accepted: April 21, 2022

DOI:
10.2174/1570159X20666220507022701

Abstract: Blood-Brain Barrier (BBB) acts as a highly impermeable barrier, presenting an impediment to the crossing of most classical drugs targeted for neurodegenerative diseases including Parkinson's disease (PD). About the nature of drugs and other potential molecules, they impose unavoidable dose-restricted limitations eventually leading to the failure of therapy. However, many advancements in formulation technology and modification of delivery approaches have been successful in delivering the drug to the brain in the therapeutic window. The nose to the brain (N2B) drug delivery employing the nanoformulation, is one such emerging delivery approach, overcoming both classical drug formulation and delivery-associated limitations. This latter approach offers increased bioavailability, greater patient acceptance, lesser metabolic degradation of drugs, circumvention of BBB, ample drug loading along with the controlled release of the drugs. In N2B delivery, the intranasal (IN) route carries therapeutics firstly into the nasal cavity followed by the brain through olfactory and trigeminal nerve connections linked with nasal mucosa. The N2B delivery approach is being explored for delivering other biologicals like neuropeptides and mitochondria. Meanwhile, this N2B delivery system is associated with critical challenges consisting of mucociliary clearance, degradation by enzymes, and drug translocations by efflux mechanisms. These challenges finally culminated in the development of suitable surface-modified nano-carriers and Focused- Ultrasound-Assisted IN as FUS-IN technique which has expanded the horizons of N2B drug delivery. Hence, nanotechnology, in collaboration with advances in the IN route of drug administration, has a diversified approach for treating PD. The present review discusses the physiology and limitation of IN delivery along with current advances in nanocarrier and technical development assisting N2B drug delivery.

Keywords: Parkinson's disease, blood-brain-barrier, nose-to-brain delivery, intranasal, nano-carriers, nano drug delivery.

1. INTRODUCTION

Parkinson's disease (PD) is the second most common, serious neurodegenerative disorder (NDD) following Alzheimer's disease (AD) which affects millions of people around the world [1]. PD is a complex, age-related, progressive neurodegenerative disorder characterized by movement impairments due to the loss of dopaminergic neurons of substantia nigra pars compacta. The loss of dopaminergic neurons diminishes the dopamine (DA) level in the striatum and subsequently leads to motor symptoms such as resting tremors, muscle rigidity, and akinesia/bradykinesia. At the same time, the non-motor symptoms involve anxiety, depression, apathy, cognitive dysfunction, and digestive disorders that might be due to loss of DA or other factors. PD is a multifactorial disease caused by various environmental triggers and could also be due to genetic risk factors. Environmental factors include exposure or consumption of pesticides, herbicides, industrial chemicals, or various toxins like 1, 2, 3, 6-methyl-phenyl-tetrahydropyridine (MPTP), which has been predominately found in PD patients. In 5-10% of PD patients, the cause is genetic where mutations in alpha-synuclein, phosphatase, and tensin homolog (PTEN)-induced kinase 1(PINK1), parkin, leucine-rich repeat kinase 2 LRRK2, ubiquitin-protein hydrolase (UCHL1), and protein deglycase (DJ1) genes were reported. Further, the

*Address correspondence to these authors at the Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research (NIPER)-Hyderabad, Balanagar, Hyderabad, TS-500037, India; Tel: 040-23073741; Fax: 040-23073751; E-mail: sbsingh.dipas@gmail.com (S.B.S), E-mail: dkkhatri10@gmail.com (D.K.K.)

[#]*These Authors contributed equally to this work.*

1875-6190/23 $65.00+.00

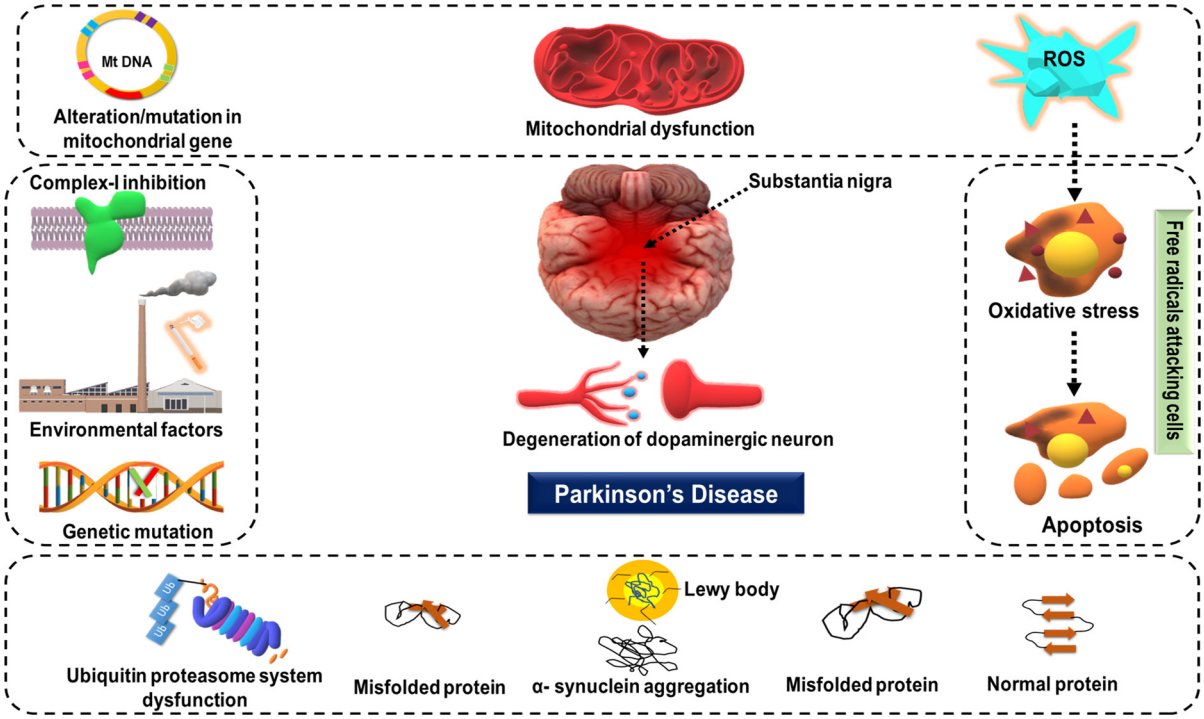

Fig. (1). Schematic representation of the pathophysiology of Parkinson's disease. Various factors are responsible for the pathophysiology of PD. A genetic mutation causes impairment in the ubiquitin-proteosome system, which then leads to misfolding of alpha-synuclein protein. This misfolded protein accumulates in the form of Lewy bodies. Complex-1 inhibition by environmental toxicants and mutation in the mitochondrial gene causes mitochondrial dysfunction. Increased ROS generation leads to an increase in oxidative stress, which activates various apoptotic caspases followed by apoptosis. All these factors collectively result in the degeneration of dopaminergic neurons in substantia nigra. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

pathogenesis of PD (Fig. **1**) involves the misfolding of proteins, excitotoxicity, apoptosis, oxidative stress, and mitochondrial dysfunction.

In PD the ubiquitin-proteasome system, molecular chaperones, and autophagy lysosomal pathway are actively involved in the degradation of misfolded proteins. Failure or impairment in such protein degradation systems led to the accumulation of misfolded proteins, which form insoluble aggregates and interfere with neuronal communications. PD involves specially misfolding of alpha-synuclein proteins, which form insoluble aggregates of Lewy bodies that get accumulated into the dopaminergic neurons and affect the neuron-to-neuron transmission [2]. Meanwhile, there are many classical antiparkinsonian agents which possess therapeutic potential at par excellence with few limitations. They need to pass through the central nervous system (CNS) barriers to get access to the target brain region, and hence the latter is one of the major challenges with PD therapy [3]. The other limitation is also the peripheral metabolism of the drugs such as levodopa, and hence to reach the therapeutic concentration doses need to be increased which can cause dose-related toxicity. However, the CNS barrier remains the main hurdle, especially in the case of brain-targeted drug delivery.

2. CHALLENGES ASSOCIATED WITH BRAIN

The CNS is protected by two main barriers, namely blood-brain-barrier (BBB) and blood-cerebrospinal fluid-barrier (BCSFB). These lipoidal barriers are meant to control the passive entry of any foreign substance including therapeutic agents. Both barriers block the entry of almost all the drugs, presenting an uphill journey for CNS drug delivery. Therefore, about 98% of drugs cannot enter the brain and, hence are unable to treat PD and other NDDs like AD [4]. Some lipophilic drugs which have a partition coefficient of 1.5-2.7 and less than 600 Dalton molecular weight can cross barriers to enter the brain [5].

2.1. Blood-Brain-Barrier (BBB)

The BBB mainly consists of specialized endothelial cells and perivascular elements such as astrocytes and pericytes. The blood capillaries of the CNS present different architecture compared to the periphery. Moreover, all the capillaries present in the spinal cord and the brain of vertebrates are deficient in small pores, which are responsible for the movement of solutes from blood circulation to other organs. This specialized arrangement of brain capillaries with neuroglial cells forms a tight junction and blocks the passage of drugs [6].

2.2. Blood-Cerebrospinal Fluid-Barrier (BCSFB)

It is the second major barrier of CNS following BBB, which is present between circulation and cerebrospinal. It consists of the choroid plexus and the arachnoid membrane, and it has a tight epithelial junction that prevents the free passage of drugs and other molecules to CSF. The BCSFB is a less significant barrier than BBB because it has 1000 times less surface than BBB. It restricts the entry of various solutes into CSF and protects the CSF from various types of toxins, and foreign agents [6].

Meanwhile, other delivery routes to the brain like intracranial, intraventricular, intracerebral, spinal, *etc.* are invasive and could precipitate serious adverse effects which include nerve damage, neuropathy, stroke, susceptibility to infections, intracranial hypertension, *etc.* Self-assembled amphiphilic core-shell nanocarriers in line with the modern strategies for brain delivery [7]. Therefore, various alternative approaches are being investigated which could win over many limitations discussed so far. The IN route is one such alternative drug delivery approach that has shown many advantages over others. The IN route delivers drugs successfully to the brain bypassing the BBB, as many recent studies of Nose-to-Brain (N2B) drug delivery has been successful in treating NDDs [8]. Meanwhile, it needs confirmatory data from the clinical studies to get acceptance for future use on clinical premises. Therefore, in the present review, firstly, we have tried to understand the PD current therapies with their advantage and limitation and what advantages N2B delivery offers over later and recent advancements with special emphasis on N2B delivery. We also tried to discuss how by using nano polymer-carriers we can increase the efficacy of IN drug delivery.

3. CURRENT THERAPY AND LIMITATIONS

The main underlying cause of PD is the imbalance between the concentrations and activity of the neurotransmitters involving acetylcholine and DA. Hence, most of the therapies are targeted to maintain the balance either by increasing the DA or by decreasing acetylcholine. They mainly provide symptomatic relief but lack curative potential as they are unable to abolish the PD progression. Currently available medications are mainly classified into two classes subsequently: (A) Dopaminergic drugs and (B) Non-dopaminergic drugs based on their mode of action. A summary of the current medication and its limitations are briefly represented in Table **1**.

3.1. Dopaminergic Drugs

These are the class of drugs that are mainly concerned with enhancing the DA activity in the substantia nigra. levodopa or L-DOPA acts as the hallmark drug and acts as a "gold standard treatment" for PD.

Bromocriptine, ropinirole, and pramipexole also come under the dopaminergic class of drugs. Principally, they act on the dopaminergic receptors present in the brain.

Tolcapone and entacapone are chiefly known as catechol-o-methyl transferase (COMT) inhibitors. The metabolic role of the COMT enzyme is the biotransformation of DA into 3-methyltyramine (3-MT), which is further oxidized to metabolite homovalinic acid (HVA). The mode of action of these drugs is to inhibit the COMT enzyme reversibly, which eventually facilitates increasing the bioavailability as well as inhibiting the metabolism of levodopa in the periphery.

Monoamine-oxidase (MAO-B) inhibitors mainly selegiline and rasagiline act as dopaminergic drugs. The mode of action of these drugs involves irreversible inhibition of the MAO-B enzyme which is involved in the biotransformation of DA to 3,4-dihydroxyphenylacetic acid (DOPAC).

3.2. Non-Dopaminergic Class of Drugs

The drugs under this class do not necessarily enhance DA activity but possess different modes of action other than action on DA. Amantadine is an antiviral drug that acts as an antagonist on the NMDA- receptors of glutamate, thus inhibiting the neurotransmission pathway of the glutamate involved in PD on the basal ganglia of the brain. Centrally acting anticholinergics also come under the non-dopaminergic class of drugs. Benzhexol and benztropine are two drugs of this class that mainly act as an antagonist and by inhibiting the overactivity of acetylcholine, they provide symptomatic relief. These drugs have been used as primary choices in drug-induced parkinsonism [9, 10]. Zolpidem specifically acts as an agonist at the GABA receptor, and it can be used with levodopa for reducing the on/off symptoms [11].

3.3. Surgical Procedure for PD Treatment

Other than the dopaminergic and non-dopaminergic classes of drugs, invasive treatments are also available for treating PD.

Deep Brain Stimulation (DBS) has been a novel and acceptable surgical technique to treat the primary symptoms occurring in PD along with the reduction in the need for levodopa therapy, which is having a major limitation of on/off symptoms. The advantage of this system is that it helps in stimulating the deep areas of the brain continuously, generally the thalamus or globus pallidus internal, which became ineffective or non-functional in PD. DBS have their share of limitations besides being invasive, and partially efficacious with risk factors such as chances of major infection, hemorrhage along with complications in patients with stroke. Meanwhile, patients do not necessarily suffer from motor symptom throughout the day, and hence DBS can disturb normal physiology, too [12].

Other than DBS, pallidotomy and thalamotomy techniques are used as surgical techniques for treating PD. The pallidotomy is primarily the destruction of a particular part of the globus pallidus to reduce resting tremors, muscular rigidity, and bradykinesia occurring in PD. Thalamotomy has largely involved the destruction of the thalamus with an intent to disrupt the internal connection between the ganglia and cortex involving motor activities [12].

3.4. Stem-Cell Transplantation Therapy

This is also a promising approach to treat PD when there is almost less response to levodopa therapy. The technique mainly involves the transplantation of embryonic stem cells (mainly adrenal medullary DA cells) to improve the motor activity defects occurring in PD patients [13]. As we have seen so far, the updated therapy of PD and how their associated disadvantage limits their therapeutic potential. Therefore, further, we are going to discuss what N2B delivery has to offer in advancing the therapy.

4. NOSE-TO-BRAIN DELIVERY

The IN route of drug administration comes out as a promising approach to delivering the drug directly into the central nervous system (CNS), bypassing the BBB. The N2B delivery possesses several advantages over others. However, this route also has a few limitations; which include mucociliary

Table 1. Current therapies and their related limitations.

Drug Name	Mode of Action	Salient Features	Limitations	References
Levodopa/L-DOPA	D2 receptor interaction.	✓ Metabolic fate of L-DOPA comprised of biotransformation into dopamine in presence of l-amino acid decarboxylase (AADC). ✓ Treatment of the early symptom of the disease involving muscle rigidity, bradykinesia, and posture disability.	✓ 2-3% of L-DOPA only reach the brain due to peripheral conversion of dopamine in presence of DOPA decarboxylase. ✓ On"/ "Off" effect. ✓ Effect on heart: Asymptomatic postural hypotension and arrhythmia. ✓ Effect on GIT: Anorexia, vomiting, and nausea.	[132-135]
Dopaminergic agonist 1. Bromocriptine	D2 receptor agonist and D1 receptor partial agonist.	✓ Binding on D2 receptor results in inhibition of adenylyl cyclase and, decreases cAMP levels. ✓ Binding on D1 receptor leads to adenyl cyclase activation with increased levels of cAMP.	✓ Constipation, vasospasm, peptic ulcers along with vascular vasospasm in the periphery.	[136]
2. Ropinirole	D2 receptor agonist.	✓ Inhibition of adenyl cyclase and decrease in the cAMP level. ✓ Treatment of restless leg syndrome.	✓ Postural hypotension, dyskinesia, along with somnolence.	[137]
3. Pramipexole	D3 receptor agonist.	✓ Decreased the cAMP levels due to inhibition of adenylyl cyclase.	✓ Nausea vomiting and somnolence.	[138]
COMT Inhibitors Tolcapone and Entacapone	Reversible inhibition of COMT enzyme which enhances the bioavailability of Levodopa metabolism.	✓ The metabolic significance of the catechol -o-methyltransferase (COMT) enzyme is biotransformation of DA into 3-methyltyramine (3-MT). ✓ Tolcapone has a longer half-life and more potency than Entacapone.	✓ Hepatotoxicity, Hallucinations, and Dyskinesia.	[139-142]
Monoamine oxidase (MAO-B) inhibitors Selegiline and Rasagiline	Irreversible inhibition of the MAO-B enzyme which compromises biotransformation of DA to3,4 dihydroxy- phenylacetic acid (DOPAC).	✓ Mainly used to treat idiopathic Parkinsonism ✓ Rasagiline action is more potent than Selegiline.	✓ Nausea, vomiting, and anxiety.	[143-145]
NMDA receptor antagonist Amantadine	NMDA receptor antagonist of glutamate.	✓ Neurotransmission pathway inhibition of glutamate on basal ganglia in PD patients. ✓ Symptomatic relief to PD patients. ✓ In combination with L-DOPA.	✓ Livedo-reticularis.	[9, 146, 147]
Centrally acting anticholinergics Benzhexol and Benztropine	Acetylcholine antagonist.	✓ Inhibition of overall acetylcholine overactivity.	✓ Confusion, dry mouth, urinary retention, and constipation.	[148, 149]
GABA receptor agonist Zolpidem	GABA receptor agonist.	✓ Combination therapy with L-Dopa to inhibit on/off symptom.	✓ Incidence of PD on long term use. ✓ Addictive potential.	[150, 151]

(Table 1) contd….

Drug Name	Mode of Action	Salient Features	Limitations	References
Surgical methods 1. Deep brain stimulation	Continuously stimulating deep brain areas like the thalamus or globus pallidus which turn less effective in PD.	✓ Comprises of implantation of two fine electrodes on both sides of the chest usually under the collar bone, which can be on/off as per need with remote.	✓ Complications with patients having hemorrhage and/or infections. ✓ Tumor potential.	[152-154]
2. Pallidotomy	Destruction of a selective part of the brain involving Globus-Pallidus.	✓ Symptomatic relief of PD involving resting tremors, muscular rigidity, and bradykinesia.		[155, 156]
3. Thalamotomy	Destruction of a selective part of the thalamus.	✓ To disrupt the internal connection between ganglia and cortex involving motor activity.		[156]
4. Stem cells transplantation therapy	Transplantation of embryonic stem cells.	✓ Improvement of motor activity.		[13, 157, 158]

clearance, enzymatic degradation by nasal enzymes, nasal irritation, and lesser surface area for absorption of drugs compared to the GI tract [14]. To overcome the above limitations, the N2B delivery route is being improved by employing various novel approaches. Before discussing all the approaches, let's have a look at the anatomy and neural connection between the nose and neuron.

4.1. Anatomy of the Nasal Cavity

The nose has a nasal passageway of 12-14 cm deep with a volume of around 16-19 cm^3 and a surface area of about 180 cm^2 with two cavities divided by a nasal septum [15]. The nasal cavity is located between the oral cavity and the skull base. It is lined with a mucous layer and hair. The nasal cavity mainly consists of three regions which include the vestibule, respiratory region, and olfactory region. The nasal vestibules are two entry points which cover the most anterior part of the nasal cavity. It is enclosed by the cartilage of the nose and lined by stratified squamous and keratinized epithelium. The respiratory region of the nasal cavity regulates functions such as protection, humidification, filtration, and elimination of debris. This region consists of four types of cells, *i.e.*, goblet, basal, ciliated, and non-ciliated columnar cells, which regulate the exchange of electrolytes between cells and cilia. It is lined by ciliated pseudostratified epithelium, interspersed with mucus-secreting goblet cells. The olfactory region is located in the superior region of the nasal cavity and is lined by olfactory cells with olfactory receptors (Fig. **2**) [16, 17].

4.2. Pathways and Mechanisms for Nose-to-Brain Delivery

In the last few decades, N2B delivery gaining promising attention, and it has its unique mechanism of drug delivery into the brain. The nasal cavity has olfactory and trigeminal nerves, which are linked between nasal mucosa and the brain. When the therapeutic agent is administered by IN route, it first enters the nasal cavity, then it reaches the nasal mucosa, and finally, it gets delivered to the brain by olfactory as well as trigeminal nerves. This transport of therapeutic agents from the nasal mucosa to the brain involves direct transport, unlike the vascular pathway where the drug delivery takes place *via* indirect transport. The paracellular/extracellular and transcellular mechanisms are the two main mechanisms for drugs to get absorbed across nasal mucosa and nasal barriers to enter the brain. The paracellular mechanism involves the transport of water-soluble agents, which get diffused into the various parts of the brain across the nasal epithelium barrier. Drugs cross through the tight junction between adjacent neural cells to enter the brain, and the permeability of medications is dependent on the presence of a tight junction at the nasal epithelium barrier. It is also known as the extracellular pathway of drug transport (Fig. **2D**). The transcellular mechanism adopts the lipoidal route of transport, which transports the lipophilic agent from the nasal cavity to the brain. It is an intracellular pathway across the nasal epithelium that involves the uptake of the drug into neurons by endocytosis or pinocytosis [16, 18, 19].

4.3. Olfactory Nerve Pathway

As previously discussed, this pathway involves the direct transport of pharmacological agents from the nasal cavity to the brain *via* the olfactory nerve (Fig. **2A**). The olfactory nerve starts from the nose and ends in the primary and secondary olfactory cortex, an area associated with the sense of smell. The olfactory neuroepithelium is not protected by BBB, and it is the only region of the brain which is in contact with the external environment. When a drug is administered intranasally, it enters the nasal cavity, then through the olfactory epithelium, it reaches the brain by the olfactory nerve pathway further, dividing into the intracellular pathway and extracellular pathway. The intracellular pathway involves the uptake of the drug into olfactory sensory neurons across the olfactory epithelium, which follows the axonal transport through the cribriform plate of the ethmoid bone and reaches the olfactory bulb wherefrom the drug is further distributed to the CNS. By this pathway, the drug takes hours to days to reach various regions of the brain. In the extracellular pathway of drug transport, the drug gets transported between the gaps of neurons and mainly involves bulk flow transport to enter the olfactory bulb. From the olfactory bulb, it enters different parts of the brain by diffusion or convection. Compared to the intracellular pathway, this pathway requires some minutes for the drug to reach the brain [16, 18-20].

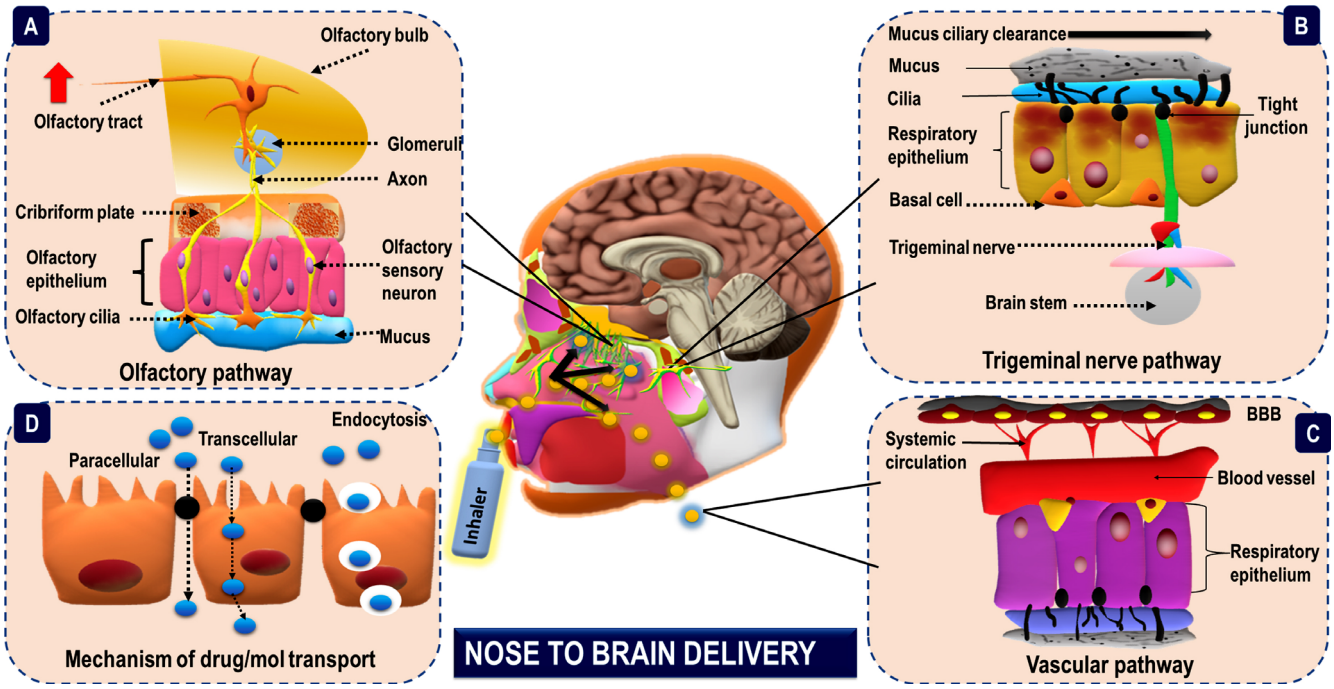

Fig. (2). Pathways and their favourable mechanisms involved in the N2B drug delivery. (**A**) The olfactory pathway involves the transport of drugs from the nasal mucosa to the brain *via* the olfactory nerve. (**B**) Trigeminal nerve pathway. Drug from nasal mucosa entered into the respiratory epithelium then reaches the brain stem through the trigeminal nerve. (**C**) Vascular pathway of drug transport. In this pathway, drugs are distributed to the systemic circulation, and then crossing BBB reaches the brain. (**D**) Mechanism of drug transport. It involves two types of drug transport *i.e.*, paracellular and transcellular mechanisms. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

4.4. Trigeminal Nerve Pathway

It also involves the direct transport of pharmacological agents from the respiratory region of the nasal cavity to the brain by the trigeminal nerve (Fig. **2B**), which innervates the respiratory and the olfactory epithelium through its ophthalmic and maxillary branches terminating in the brain. It involves an intracellular as well as an extracellular pathway for the transport of drugs from the nasal mucosa. The drug in the respiratory and olfactory regions undergoes exocytosis or it gets diffused from the nasal mucosa of the nasal cavity to trigeminal nerve endings and transported to various parts of the brain [21, 22].

4.5. Vascular Pathway

The above two neuronal pathways involve direct delivery of intranasally administered drugs, whereas this pathway, unlike trigeminal and olfactory, delivers the drug indirectly to CNS. In this pathway, first drug is administered by IN route, then it enters the nasal cavity where it gets absorbed into the systemic circulation (Fig. **2C**). From systemic circulation, it reaches to brain by crossing BBB. In the N2B delivery, the neuronal pathways are major routes for the drug to access the brain, while the vascular pathway is a minor route for the transportation of drugs across the BBB. It is mainly under investigation for other wide range of indications such as migraine, headache, infection prevention, pain management, hormone replacement therapy, smoking cessation, and emergency therapy, like as epileptic seizures [23, 24].

4.6. Effect of Cerebrospinal Fluid Pathway & Glymphatic System on Nose to Brain Delivery

In direct drug delivery from N2B *via* the olfactory and trigeminal neural pathway, drugs enter the brain *via* CSF. Briefly, the CSF enters the brain parenchyma along the paraarterial spaces surrounding the penetrating artery and interchanges the materials with interstitial fluid (ISF). The cleared ISF from parenchyma along the para venous route recirculates in the subarachnoid spaces. The circulation of CSF & ISF makes the glymphatic system of the brain which helps in the clearance of many wastes material like β-amyloid peptide, α-synuclein [25, 26]. Now we can say that the glymphatic system can majorly affect the PK of drugs in the brain and the efficiency of N2B delivery. The glymphatic system is known to be very active during sleep and under general anaesthesia. There are studies where the effect of anaesthesia on the efficiency of drug delivery to the brain *via* N2B has been evaluated however studies for PD-therapy still need to be carried out. Meanwhile, in one such study caffeine, N2B delivery from CSF, and its extracerebral clearance were enhanced under anesthesia compared to under a conscious state, possibly due to the activation of glymphatic system [27]. In another similar study use of Dexmedetomidine, a α2-adrenergic agonist sedative enhances the brain delivery of intrathecally administered therapeutic by manipulating the glymphatic system [28], however, whether the same would be beneficial for IN delivery needs to be explored.

5. CHALLENGES IN NOSE-TO-BRAIN DELIVERY: IN THE PERSPECTIVE OF PD

5.1. Challenges Associated with the Nasal Cavity

Despite the many advantages offered by IN route, it may also experience many hurdles while passing through the nasal cavity. Drugs can be expelled out by mucociliary clearance, degraded by enzymes, transported to various regions, and/or thrown out of the nasal cavity by the efflux system. Later could cause a loss of substantial drugs before reaching its target site.

5.2. Mucociliary Clearance

The nasal mucosa plays an important role in natural defense by entrapping the microbes and foreign bodies from inhaled air. Movements of mucus along with cilia expelled out the foreign particles and protected the nasal cavity. In the same way, mucociliary clearance can remove the drug from the nasal cavity, further causing a decrease in the residence time of the drug in the nasal cavity [29]. Meanwhile, mucoadhesive agents can be administered to overcome the mucociliary clearance and which would help in increasing the residence time of the drug in the nasal cavity [30, 31]. A recent development in computational analysis of a 3D mucociliary clearance model can help in predicting drug uptake and designing IN drug delivery [32].

5.3. Enzymatic Degradation

The delivery of peptides and proteins through nasal mucosa can be restricted by peptidase and protease enzymes, respectively. Similarly, several other enzymes such as aldehyde dehydrogenase, epoxide hydrolases, esterases, and glutathione s-transferases are present in the nasal cavity and are involved in the metabolism of many drugs. Small molecules like levodopa employed in the treatment of Parkinson's disease suffer from extensive first-pass metabolism and hence, possess a short half-life. Various enzyme inhibitors can be administered to inhibit specific enzymes which ultimately inhibit enzymatic degradation [33]. Various pro-drug strategies in targeting the BBB, diminishing the first-pass metabolism, enhancing pharmacokinetic stability, *etc.* have been explicitly described in an excellent review by Cacciatore *et al.* [34].

5.4. Transporters and Efflux System

The respiratory and olfactory region of the nasal cavity has several transporters that have developed resistance to multiple drugs and are actively involved in the transportation of various lipophilic and amphiphilic substances. The p-glycoprotein is expressed by ciliated epithelia and submucosa of the olfactory region of the nasal cavity. Latter is involved in the efflux of drugs directly out of nasal mucosa to the lumen, leading to a decrease in the drug absorption [35, 36].

6. NANOCARRIERS FOR NOSE-TO-BRAIN DELIVERY

When a drug is administered in its native form for N2B delivery, it faces many hurdles as explained earlier. To overcome the above defined issues, drugs could be formulated as various suitable nanoformulations with the aid of different nano-carriers (as shown in Table **2**). Nano-formulations prevent drug degradation in the nasal cavity, improve drug transport across nasal mucosa, and increase intranasal brain delivery of drugs, ultimately helping in enhancing the treatment for brain disorders like PD.

6.1. Chitosan Nanoparticles

Chitosan is a mucopolysaccharide and deacetylated form of chitin that is suitable for N2B drug delivery. It has been used more commonly for the preparation of various nanoparticles (NPs) because it bears properties like good stability, less toxicity, biodegradability, mucoadhesiveness, and better biocompatibility [37, 38]. The D-glucosamine units of chitosan are positively charged containing amine groups and mucin a negatively charged containing sialic acid groups (Fig. **3**). The ionic interaction between such oppositely charged groups results in mucoadhesion. This mucoadhesion of chitosan successfully increases the residence time of the drug into the nasal mucosa and subsequently improves the diffusion of drugs across the nasal mucosa of the nasal cavity [39, 40]. In one of the research studies, pramipexole dihydrochloride-loaded chitosan NPs equivalent to 0.3 mg/kg intranasally, which were prepared by ionic gelation method, was administered intranasally into the PD model of rat. This N2B delivery of chitosan NP-containing drugs successfully increased DA concentration in the brain compared to other treatment groups resulting in an enhancement in the activity score, glutathione, and catalase activity, and reduction in catalepsy score [41]. Additionally, in another study carried out in the rat PD model by Sridhar *et al.*, chitosan was used for the preparation of selegiline NPs. After IN administration of selegiline NPs, the concentration of selegiline equivalent to 1 mg/kg was found to be 20-fold higher in the brain compared to oral administration [42]. Apart from these, the intranasal administration of ropinirole hydrochloride-loaded chitosan NPs evaluated by gamma scintigraphy in swiss albino rats tagged with 99mTc revealed increased brain distribution of the drug [43]. Kumar *et al.* incorporated leucine-enkephalin (Leu-Enk), a neurotransmitter within chitosan nanoparticles for the N2B delivery. Enhanced peptide encapsulation efficiency and loading capacity (78.28 ± 3.8% and 14 ± 1.3%), with size, PDI and cationic surface charge - 443 ± 23 nm, 0.317 ± 0.17 and +15 ± 2 mV respectively. The apparent permeability coefficient (P_{app}) of Leu-Enk released from nanoparticles across the porcine nasal mucosa was determined to be $7.45 \pm 0.30 \times 10^{-6}$ cm s^{-1}. Permeability of Leu-Enk released from nanoparticles was augmented by 35-fold compared to Leu-Enk solution. Fluorescent microscopy of brain sections of mice showed a high accumulation of fluorescent marker NBD-F labeled Leu-Enk when administered nasally by chitosan nanoparticles. Furthermore, substantial improvement in the brain uptake resulted in significant improvement in the anti-nociceptive effect of Leu-Enk was proved by hot plate and acetic acid-induced writhing tests ($p < 0.05$). Molding chitosan and effectively utilizing its beneficial properties could lead to substantial enhancement in therapeutic efficacy in brain disorders [44].

6.2. Polymeric Nanoparticles

The difficulty in penetrability of the BBB imparted by the tight junctions and efflux proteins limit the traversing and

Table 2. Various nano-carriers used for nose-to-brain delivery in Parkinson's disease.

Nano-Carrier	Method of Preparation	Name of Drug	Animal Model	Outcome	References
Chitosan Nanoparticle	Ionic gelation	Pramipexole Hydrochloride	Rat	N2B delivery of Pramipexole dihydrochloride loaded chitosan treated group showed significantly highest dopamine concentration (97.38±3.91 ng/g tissue) compared to other treatment groups.	[41]
PLGA Nanoparticle	Nanoprecipitation	Rotigotine	Mice	IN the administration of Rotigotine loaded PLGA nanoparticles showed a higher concentration of Rotigotine in the striatum than in plasma.	[45]
Chitosan Nanoparticle	Ionic gelation	Selegiline	Rat	After IN administration of Selegiline nanoparticles, the concentration of Selegiline was found to be 20 fold higher in the brain compared to oral administration.	[159]
PLGA Nanoparticle	Double emulsification	Rasagiline	Rat	IN PLGA nano formulation of Rasagiline had given a promising PK profile and provided effective treatment for PD as compared to IN solution form of Rasagiline.	[46]
Mucoadhesive Nanoemulsion	Aqueous titration	Rotigotine	Goat nasal mucosa	The Rotigotine mucoadhesive nanoemulsion had a better permeability through nasal mucosa (85.23±0.39%) compared to Rotigotine nano emulsion (65.25±0.13) at 4 hours.	[53]
Nanoemulsion	Aqueous titration	Vitamin E loaded Naringenin	Rat	N2B delivery of Vitamin E loaded Naringenin nanoemulsion improved muscle coordination, grip strength and swimming activity of 6-OHDA induced PD model.	[50]
Nanoemulsion (o/w)	Spontaneous emulsification method, followed by high-pressure homogenization	Resveratrol-loaded vitaminE	Rat	The improved antioxidant activity, increased SOD,GSH and decreased MDA level. Improved brain/blood ratio of the drug. Histopathological studies showed decreased degenerative changes.	[160]
Lipid nanoparticle	Melt-emulsification	GDNF	Mice	N2B delivery GDNF Lipid nanoparticles significantly improved locomotor activity and motor recovery in MPTP induced PD model.	[161]
Mucoadhesive Nanoemulsion	Response surface methodology	Ibuprofen	Mice	MPTP induced Parkinsonian condition significantly reduced striatal dopamine level to 29.92% which was enhanced to 58.21% after IN delivery of Ibuprofen loaded mucoadhesive nanoemulsion	[52]
Nano-Emulsion	High energy emulsification	Selegiline	Rat	IN administered Selegiline nanoemulsion showed high dopamine level (16.61±3.06ng/ml) as compared to rats treated with Haloperidol (8.59±1.00ng/ml)	[51]

(Table 2) contd….

Nano-Carrier	Method of Preparation	Name of Drug	Animal Model	Outcome	References
Chitosan Nanoparticle	Ionic gelation	Ropinirole Hydrochloride	Rat	IN administered Ropinirole hydrochloride loaded Chitosan nanoparticles showed higher concentration in the brain at all the time points compared to IN. Ropinirole Hydrochloride administration.	[162]
Micellar thermo-responsive hydrogel	Solvent evaporation	Rotigotine	Rat	Compared to intravenous route, accumulation in the olfactory bulb, cerebrum, cerebellum and striatum was 276.6%, 170.5%, 166.5% and 184.4%, respectively.	[163]
TAT functionalized micelles	Thin film dispersion method	-	Rat	TAT functionalization diminished the retention of micelles in the nasal cavity and brain uptake *via* trigeminal nerve was observed.	[164]
Liposomes	Thin film dispersion	Glial cell line-derived neurotrophic factor	Rat	A substantially greater neurotrophic effect was observed with increase in tyrosine hydroxylase positive neurons with greater neuroprotective ability determined by TH immunostaining was observed.	[165]
Solid-lipid Nanoparticle	Emulsification solvent diffusion	Ropinirole Hydrochloride	Mice	Nasal formulation of solid lipid nanoparticles of Ropinirole hydrochloride significantly reduced tremors against the marketed oral formulation.	[57]

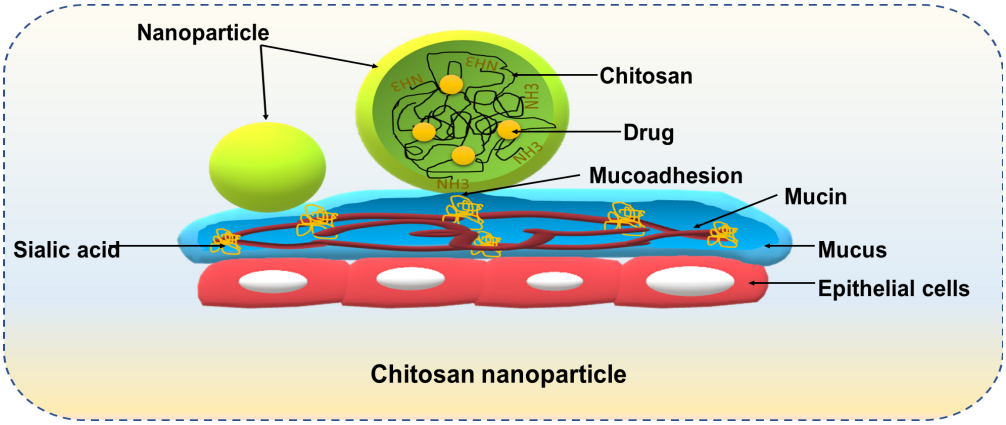

Fig. (3). Structural overview of chitosan nanoparticle. Positively charged groups of chitosan nanoparticles interact with negatively charged groups of mucin of the nasal mucosa. This interaction enhances the residence time of nanoparticles in the nasal cavity. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

therapeutic efficacy in numerous central nervous system disorders. Many intra-nasally administered drugs get degraded in the nasal cavity and are unable to reach the brain. The nano-formulation of polymeric NPs inhibits the active degradation of drugs and enhances drug delivery to the brain. In the study of Chenchen *et al.*, it was observed that IN administration of rotigotine loaded NPs in the PD mice model significantly enhanced striatum rotigotine concentration compared to plasma rotigotine concentration [45]. Similar in the line, the rasagiline-encapsulated PLGA NPs were prepared by double emulsification solvent evaporation method" (Fig. **4a**). Then they were administered by IN route into the rat model of PD. The result has given a promising pharmacokinetic profile and provided effective treatment for PD as compared to IN solution form of rasagiline at 10 mg/kg dose [46]. Tan and co-workers linked apomorphine to phenyl boronate functionalized polycarbonate nanoparticles which showed substantial brain accumulation and pH responsive release. Encapsulation also prevented apomorphine oxidation [47].

6.3. Nanoemulsion and Mucoadhesive Nanoemulsion

Nanoemulsion (NE) is a kinetically stable biphasic emulsion consisting of two immiscible liquids, *i.e.*, oil and water(o/w). It is stabilized by the aid of surfactants and consists of dispersed droplet sizes in the range of 20-500 nm [48]. O/W and water-in-oil (w/o) are two main types of NEs

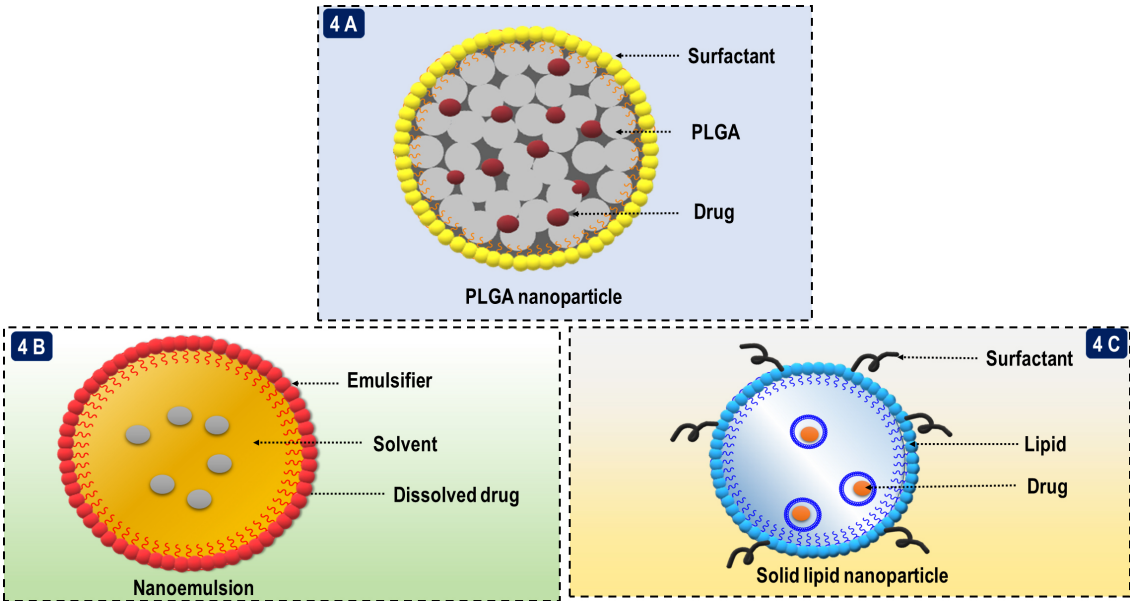

Fig. (4). Structural overview of (**A**) PLGA nanoparticles, (**B**) Nanoemulsion, and (**C**) Solid-lipid nanoparticles. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

(Fig. **4b**) which can be prepared by either high emulsification or low emulsification method. It is also known as a submicron emulsion, mini-emulsion, or ultrafine emulsion [49]. A study has shown Vitamin E-loaded naringenin NE improved muscle coordination, grip strength, and swimming activity in the 6-OHDA induced PD model following the IN route of administration [50]. Similarly, the selegiline NE showed a high dopamine level (16.61 ± 3.06 ng/mL) as compared to rats treated with haloperidol (8.59 ± 1.00 ng/mL) following IN administration [51]. Additionally, N2B delivery of mucoadhesive NE containing ibuprofen had enhanced striatal DA levels in the MPTP-induced parkinsonian mice model [52]. The mucoadhesive nanoemulsion of rotigotine had given better permeability through goat nasal mucosa [53]. Asshar *et al.* incorporated bromocriptine and Glutathione which possess poor oral bioavailability and absorption characteristics into a nanoemulsion for the amelioration of activity in PD. The optimized batch had 80.71 ± 2.75 nm size and zeta potential of −12.60 ± 0.10 mV with 96.00 ± 3.05 % entrapment efficiency respectively. Spherical morphology along with 3.4 times and 1.5 times enhancement in drug permeation of bromocryptine and glutathione was observed compared to a free drug suspension. Cytotoxicity studies on neuro-2a cell lines demonstrated its safety for intra-nasal delivery. Behavioral studies revealed enhanced efficacy of optimized nanoemulsion in PD using forced swimming test, locomotor activity test, catalepsy test, rotarod test, and akinesia test in Wistar rats. The outcomes of the behavioral studies revealed that BRM (0.21 mg/kg body weight) and GSH (61.67 mg/kg body weight) loaded nanoemulsion revealed substantial improvement in behavioral activities of PD (haloperidol-induced) rat model after intranasal administration [54]. Nehal and co-workers incorporated ropinirole nanoemulsion for Parkinson's disease management. *In silico* docking revealed interactions of ropinirole and thymoquinone at the receptor site of TNF-α and NF-Kβ. The globule size of optimized chitosan-coated nanoemulsion was 183.7 ± 5.2 nm with a cationic charge of 24.9 mV respectively. *In vitro* release and permeation studies exhibited 2-fold and 3.4-fold greater enhancement compared to a free drug suspension. Amelioration in the behavioral activity with enhanced brain distribution of drugs revealed the potential of nanoemulsions as carriers for N2B delivery [55].

6.4. Lipid-Based Nanoparticles

Lipid and solid-lipid NP (Fig. **4c**) are lipoidal carriers that have been successfully employed in the treatment of cerebrovascular and cardiovascular diseases. This type of nanoformulation can enter the brain to treat CNS disorders. Hernando *et.al*, prepared the GDNF loaded lipid NP by the Melt-emulsification method, and N2B delivery of this NP had shown significantly improved locomotor activity, and motor recovery in the MPTP-induced PD model [56]. Nasal formulation of solid lipid NP of ropinirole hydrochloride at 3 mg/kg intranasal dose significantly reduced tremors against the marketed oral tablet formulation at 10 mg/kg dose [57]. Sun *et al.* prepared spherical particles with good size distribution was 166.79 nm with Newtonian viscosity of 44.36 mPaS. The viscosity of post-*in situ* gel formation was 1542.19 mPaS with thixotropic behavior. The combination of matrix erosion diffusion resulted in sustained drug release. Cytotoxicity studies in normal brain cells revealed diminished cytotoxicity. Cyanine 7-ester linked solid lipid nanoparticulate in situ gel (Cy7-SLNs-ISG) localized effectively in the brain post olfactory area administration and the fluorescence was shown in the olfactory bulb, cerebellum, and striatum confirmed by *in vivo* imaging [58]. Uppuluri and co-workers circumvented various drawbacks associated with Piribedil conventional delivery like increased dosing frequency (5 tablets per day), poor bioavailability (less than 10%), GI adverse effects, *etc.* PBD-Solid lipid nanoparticles were loaded in thermo-responsive in situ gel to diminish the mucociliary clearance and enhance brain accumulation. *In vivo,* pharmacokinetic studies revealed 4-fold brain accumulation equivalent to 2 mg/kg intranasal dose at 40

μL/kg compared to a free drug resulting in 27% direct N2B uptake [54].

7. NANOFORMULATION CONSIDERATIONS

For any formulation to be efficacious, they need to be properly characterized considering the aimed delivery system whether it is oral, Intravenous, IN, *etc.* An ideal drug molecule needs to be small in terms of molecular weight *i.e*., low molecular weight (below 1000 Da), and rapid display of dissolution. In the case of a drug targeted for nasal delivery, it must reach the epithelium by traversing the aqueous mucus barrier besides avoiding mucociliary clearance. The drug can then be taken up-typically by transcellular diffusion and by avoiding P-glycoprotein-mediated efflux. Moreover, large hydrophilic biomolecules impede permeation and absorption. An ideal drug candidate or nanoparticulate system should consist of lipophilic nature for rapid uptake through olfactory nerves in the upper turbinate/olfactory bulb or the trigeminal nerve pathway *via* the nose [59]. As discussed, previously the mucociliary clearance is responsible for low membrane transport. Later, they can be overcome by using penetration enhancers or mucoadhesive agents which by interacting with mucin enhance the contact time between applied formulation and nasal mucosa [60].

7.1. Factors Affecting Nasal Delivery

Designing the nasal formulation should be efficient before going for the actual formulation procedure and their characterization, which depends on the various factors like physicochemical properties of the drug, nanoparticle excipients, targeted delivery organs such as IN, and the patient-related factor.

7.1.1. Physicochemical Properties of a Drug

7.1.1.1. Molecular Weight

The drug molecules should be of low molecular weight preferably below 1000 Da and show rapid dissolution. Higher is the molecular weight of the drug, there will be a decrease in nasal drug absorption.

7.1.1.2. Polymorphism

Polymorphism can also affect the rate of drug dissolution, solubility, and absorption through biological membranes.

7.1.1.3. Solubility and Dissolution Rate

The solubility and dissolution rate are the determining factors affecting nasal drug absorption for suspensions and powders. Drug absorption is not only affected by drug solubility but is also influenced by pharmaceutical preparation. Owing to the small size of the nasal cavity, only the limited drug volume can pass through intranasal administration. Generally, the requirement of a high dose of drugs and poorly water-soluble drugs may affect the dissolution rate. However, the aim of the good formulation is such that the small particles deposited into the nasal cavity need to get dissolved first and then absorbed.

7.1.1.4. Lipophilicity

By enhancing lipophilicity the permeation of the drug compound in the nasal mucosa can be increased. The biological membranes can be immediately crossed *via* a transcellular route by lipophilic compounds. Various lipophilic substances like buprenorphine, naloxone, testosterone, and 17α-ethinylestradiol are wholly or mostly absorbed by the intranasal route in animals.

7.1.1.5. Partition Coefficient and pKa

Basically, the unionized drug molecule is absorbed better than the ionized drug molecule conferring to the pH partition theory [61-64].

7.1.2. Physicochemical Properties of Nanoparticles

7.1.2.1. Particle Size

The particle size ranges from 5 to 10 microns and has been tried in nasal administration. Very tiny particles (below one micron) exhibit Brownian motion and exit the nasal cavity during exhalation. Particles greater than 10μm in size can be aimed for delivery in the upper pulmonary tract. Particles of less than 1μm size quickly enter the bloodstream through the intranasal route. However, compounds larger than 1000 Daltons can achieve the bioavailability of only 0.5 to 5%.

7.1.2.2. Chemical Nature

The chemical nature of a drug is the most important factor for drug absorption and in many cases, the salt formation of native drugs alters lipophilicity and ionization which further influences their absorption rate [65-67].

7.1.2.3. pH and Mucosal Irritation

Nasal irritations can be minimized when products are delivered within the slightly acidic pH range of 4.5 to 6.5. In addition, to avoid irritation, it also prevents the growth of the bacteria and enhances drug permeation [68].

7.1.2.4. Buffer Capacity

Nasal formulations are administered in vary fewer volumes ranging from 25 to 200 μL. Additionally, the different pH of the administered dose and concentration of the drug can affect the absorption of the unionized drugs. Therefore, buffer capacity is important for the maintenance of the pH *in situ* [69].

7.1.2.5. Viscosity

Greater contact time during permeation due to more viscosity produced by polymeric matrix and nasal mucosa enhance the time for permeation. The highly viscous formulation is greatly affected by the functions such as a change in the permeability of drugs and mucociliary clearance or ciliary beating [68].

7.1.2.6. Concentration, Dose, and Dose-Volume of the Drug

High formulation volume may restrict the extent of nasal absorption with the probability of draining out from the nasal cavity. 0.05-0.15 ml is the ideal use volume for nasal delivery with an upper limit of 0.20 ml [69].

7.1.2.7. Osmolarity

Shrinkage of the nasal epithelial mucosa can occur by administering iso-osmotic solutions. This results in enhanced permeation with inhibition of ciliary activity [68].

7.1.2.8. Excipients

Several excipients used in the formulation such as solubilizers, humectants, preservatives, antioxidants, *etc.* can also affect the absorption of the drug. Intranasal moisture is vital to avoid dehydration. Hence, humectants can be used particularly for gel-based nasal products for avoiding nasal irritation [70].

7.1.3. Physiological Factors

7.1.3.1. Drug Solubility in Nasal Secretions

Previous studies have shown that the mucous clearance and secretion rates are decreased at night which brings the alteration of the drug permeation. In such cases, chronokinetics comes into the picture which commands the pattern and rate of permeation [68].

7.1.3.2. Blood Flow/Supply

The nasal mucosa has a greater surface area enriched with blood supply making it an appropriate and optimum place for drug absorption. Drug absorption in the nasal cavity is affected by blood supply/flow, especially when a higher amount of drug passes through it [68].

7.1.3.3. Nasal Secretion

The mucus layer present in nasal mucosa (5 mm thick) is double layered. The tip of the cilia moves their head in a superficial blanket of gel, and they beat in their periciliary sol phase. The volume of mucous secreted throughout the nasal cavity is about 1.5-2.1 mL which results in the formation of a thin mucus layer further reducing the ciliary beating. This leads to the loss of contact with cilia compromising the mucociliary clearance which is advantageous in the case of intranasal formulations [71].

7.1.3.4. Nasal Cycle

The nasal cycle (NC) is the spontaneous congestion of one side of the nasal mucosa during the day accompanied by reciprocal decongestion of the contralateral side. In other words, the congestion resulting from increased blood supply due to parasympathetic stimulation and relaxation due to sympathetic stimulation affects the extent of drug permeation [72].

7.1.3.5. Nasal Cavity pH

The nasal cavity pH is ranging from 5.5 to 6.5 in adults and 5.0 to 7.0 in infants. Higher penetration of the molecules will occur mainly in unionized form owing to passive diffusion. The diverse range of nasal pH may affect the ionization state of the drug further increasing or decreasing the permeation of drugs. The standard pH of the formulation should be within the range of 4.5-6.5 in conjunction with ideal buffer capacity [71].

7.1.3.6. Frequency of the Mucociliary Clearance and Ciliary Beat

The mucociliary clearance includes the removal of foreign matter and particles from the nasal cavity and further preventing them from reaching the lower airways. Diminished mucociliary clearance and ciliary beating enhance the contact time between the carrier system and the mucus membrane thereby enhancing adhesivity and drug permeation.

7.1.3.7. Effect of Enzymatic Activity

Enzymatic activity in the nasal mucosa could influence the metabolic profile of drugs. For instance, at the mucosal membrane, nucleotides, and oligopeptides are degraded by proteases, nucleases, and aminopeptidases [29].

7.1.3.8. Pathological Conditions

Diseases like rhinitis, common cold, atopic rhinitis, and nasal polyposis cause mucociliary dysfunction, hypersecretion, and nasal mucosa irritation, enhance the mucociliary clearance, and drain the adhesive nanoformulations out of the nasal cavity resulting in therapeutic insufficiency of the incorporated moiety [71].

7.2. Nanoparticulate Nasal Formulation and Characterisation

As previously discussed, for ideal N2B delivery for PD, nanoparticulate must be deposited and accumulated in the upper posterior region of the nasal cavity where the olfactory epithelium is located (Fig. **5**). To achieve an optimal brain delivery sufficient nanoproduct must reach the olfactory epithelium deposition site which is situated proximally to the olfactory bulb and cribriform plate. The olfactory receptor-bearing neurons, which are thought to provide a pathway for drug transport, are interspersed among supporting cells (sustentacular cells), microvillar cells, and basal cells. Meanwhile, the dose-determining characteristics of delivery systems used to administer insulin in studies have also been disappointed. However, minimal details regarding the nasal device, spray characteristics, or deposition pattern are provided in a study demonstrating dose-dependent nose-to-brain insulin absorption. Studies using both *in vitro* nasal casts and computational modelling provide a general framework of nasal pump performance characteristics that are optimal for posterior drug deposition which is necessary for efficient nose-to-brain drug delivery.

7.2.1. Nanoparticulate Nasal Powder Formulation

Powder formulations offer advantages like liquid formulations owing to greater stability including and not requiring any preservative. The formulation of nasal powders tends to stick to the moist surface of the nasal mucosa before getting dissolved and cleared. To improve the absorption of powder formulation, the use of bio-adhesive excipients or agents is beneficial as later decelerates the ciliary action further decreasing the clearance rates. The powdered formulation has its limitations because of its moisture-sensitive characteristics, exclusivity to the particle size, particle shape, and flow properties which will impact deposition and absorption. The key challenge that is being aimed through powdered nanoformulation is to improve the inadequate residence time in the nasal cavity due to mucociliary clearance [73]. About their physiological cleaning mechanism beating of the nasal cilia touches the upper gel-like mucus layer which envelops the epithelium and clears later with a velocity of 6 mm/min towards the nasopharynx and throat. In the case of drug particles from conventional liquid formulations when deposited on the mucus surface, are removed from the nasal cavity in just about 15 min. Powder particles of formulation have shown higher resistance against the ciliary beat and the later effect is tailorable depending on the need for formulation by using the specific excipients. The excipients used in nasal

Table 3. List of polymers used in in-situ gel system for intranasal delivery in Parkinson's disease.

Polymer Used for *In-situ* gel System	Anti-Parkinson Drugs Incorporated	Preparation Methodology	Parameter Tested and Model	Advantages Obtained due to Intranasal Delivery	References
Chitosan and poloxamer 407	Levodopa	Ion Gelation	*In vitro* release studies obeying Higuchi kinetic model, drug release following Hexson-Crowell model, *In vivo* drug release study in rat.	A rapid increase in brain uptake due to increased bioavailability & drug absorption on the mucosa of the nasal cavity.	[75]
Poloxamer 407 and CMC	Amantadine	Cold method	Immortalised human nasal epithelial cell line RPMI 2650, Human nasal cast model.	Replicate drug release favouring increased brain uptake.	[103, 166]
Chitosan and HPMC (Hydroxy Propyl Methyl Cellulose)	Ropinirole	Cold method	*In vitro* release and permeation, *in vitro* cytotoxicity, nasal clearance, *in vivo* bioavailability, and brain uptake in rat.	Reported that brain uptake has increased 8.5 times than administration by i.v route, higher than intranasal Ropinirole control delivery.	[102]
Pluronic PF127 (Poloxamer 407) and HPMC K4M	Ropinirole	Cold method	*In vivo* bioavailability study in albino mice, *ex vivo* drug diffusion study.	Drug release is reported to be increased from 56% to 100% throughout 5 hours consequently,5 times increase in bioavailability in brain nasal administration as compared to IV route.	[104]
Poloxamer 407: Poloxamer 188(1:1) Carbopol 934 P and Chitosan	Rasagiline	Cold method	*In-vitro* drug release and *in-vivo* mucociliary transit time in rat, *in-vivo* pharmacokinetic behaviour in rabbit, nasal toxicity studies and brain uptake studies.	Reported that bioavailability in increased by 6 times along with rapid brain uptake than oral solution.	[105]

powder formulations would be soluble or insoluble fillers, mucoadhesive agents, or adsorption enhancers including enzyme inhibitors, among which mucoadhesive polymers appear to have the greatest potential to prolong the residence time in the nose. The polymer particles after getting in touch with nasal mucus get hydrated forming the polymer chain while the surrounding mucosa dehydrates. Further, hydrated polymers get entangled with the mucin of the mucus layer resulting in forming close contact between particles and mucosa. Latter's specific interactions with mucin and the change in mucus rheology prolong the nasal residence time. Trenkel *et al*. in their studies have evaluated the influence of powder nanoformulation on the nasal residence time by testing rheological and dynamic vapor sorption properties [74].

7.3. Mucoadhesive Properties

The nasal membrane contains glycoproteins that are capable of interacting with diverse materials [75]. PF127 gels have moderate bioadhesive force through hydrogen bonding and chain penetration effect. Bioadhesion increases with polymer concentration due to extensive bonding with glycoproteins. This detachment force was found to be desirable for the formulation to adhere to the nasal mucosal tissue easily. As shown in Tables **2** and **3**, incorporation of CNL in pluronic gel marginally increased the mucoadhesive force of the gel.

7.4. Intranasal Drug Delivery Screening

7.4.1. In vitro, In silico, Ex vivo Screening of Drug Release

The ultimate success of any formulation can only be confirmed when the latter is tested in the biological system. Hence the first biological screening can be performed at the *in vitro* level which can be carried out in immortalised cell lines such as Calu -3 [76], RPMI 2650 [77], and primary cell lines such as human nasal epithelium A431 [78], porcine respiratory [79], olfactory cells [80]. The *in vitro* cell culture has now been also succeeded in 3-D -printed nasal replicas [81]. The *in-vitro* drug release screening in primary cell culture offers the advantage of close simulation of the nasal mucosa, and the ability to be tested against the miscellaneous composition of cells in normal and diseased states. Meanwhile, they do have their limitation as the primary culture can only go to a limited number of subcultures and later faces the risk of contamination. The immortalised cell line offers the advantage of uniformity and easiness of handling and culture, but they also have their limitation in the form of undesired morphological changes, lacking the accurate physiological functionalities as time passes and passages increase. Talking about the screening appropriateness towards the specific formulation *in vitro* screening is not a correct

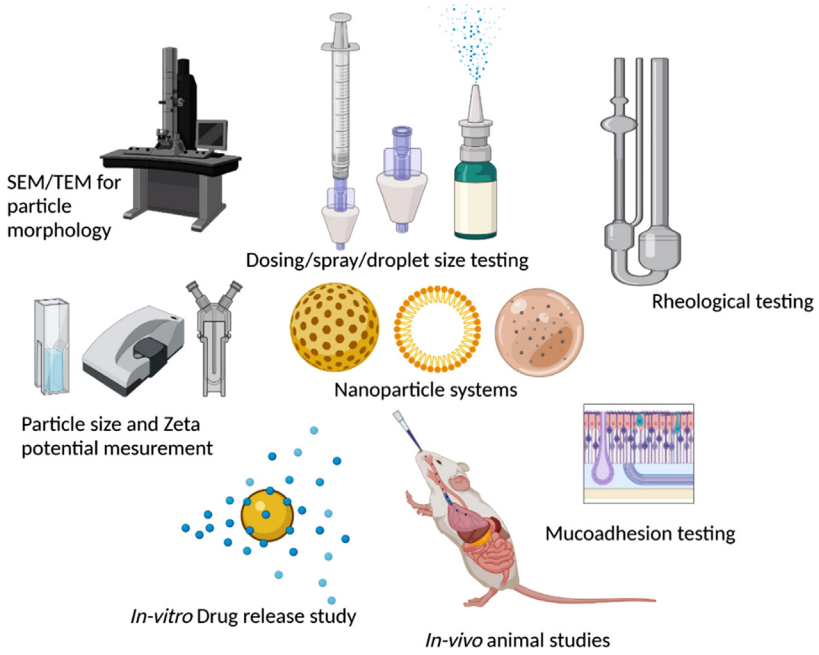

Fig. (5). Characterisation of nanomedicine system for N2B targeting for antiparkinson's disease. (*A higher resolution/colour version of this figure is available in the electronic copy of the article*).

choice for powder formulation. Later lacks reproducibility when the powder is a concern as it faces the concentration heterogeneity at cell surface layers. The method used to deposit the powder formulation could also affect the integrity of the cell [74, 82]. The 3D-printed nasal replicas are also being used as a tool to compare different nasal formulations, devices, and formulations. Later also helps in assessing the aerosol deposition pattern in the nasal cavity [83]. They suffer limitations such as one nasal cast representing one human and hence cannot be generalised to a large human population. It also lacks the inherent nature of the nasal cavity like mucociliary clearance [84-87]. Apart from *in vitro*, *in silico* models like computational particle-fluid dynamics (CPFD). It has many advantages over the classical *in vitro* model as *in the Silico* model simulation of total and regional deposition occurs and hence it offers the assessment of various nanoformulation related parameters such as particle size, velocity, airway geometry, airflow. The rate of assessment is also faster in comparison to other *in vitro* models. *In silico* delivery assume uniform, simplified nasal architecture therefore it makes calculation very challenging and complicates the spray and aerosol-generating device simulation [88, 89]. *Ex vivo* screening for nasal delivery of drug employs the excised nasal mucosa from human or animal donor, it offers real architecture of nasal tissue is intact in terms of permeation and can be used for other analyses like histological studies. Coming to the limitation latter is complicated in terms of diversity in nasal architecture like tissue thickness and enzymatic activity between different species [79, 90-92]. Some of the *in vitro* and related model used for screening of IN delivery of drug aimed at PD is also mentioned in Table **3**.

7.4.2. In vivo Studies: Parkinson Animal Models for Nose to Brain Delivery Assessment

The validation of any formulation occurs after preclinical screening is carried out in an animal model. The animal model used in the screening are generally mice, rats, monkeys. They act as a surrogate for humans where pharmacodynamic effect as drug efficacy and initial pharmacokinetics parameter like C_{max}, T_{max}, the area under the curve (AUC), % Drug targeting efficiency (DTE), N2B transport percentage (%DTP) is carried out. Some of the animal models used in the screening of antiparkinsonian effect *via* IN delivered drugs are also mentioned in Table **2**. Some of the animal models used specifically for inducing PD are 6-OHDA induced PD in Wistar rats, MPTP in mice and intranasal LPS delivery mediated PD model in mice. Additionally, optimal administration conditions and dosing regimen are important for accessing the success of IN delivery. The restricted progress in N2B delivery research is also due to these animal models as they do not represent the exact replication of intricacies and physiological conditions of human being due to interspecies variation.

An improvement in the animal model was recently described by implementing two novel methods, where *first*, IN administration of the drug was carried out under the influence of inhalation anaesthetic isoflurane and *second,* drug distribution *via* N2B delivery was assessed by using radiolabeled [^{14}C]-inulin (molecular weight: 5,000) as a model substrate of water-soluble macromolecule [93].

8. RECENT ADVANCES TO PD THERAPY EMPLOYING NOSE-TO-BRAIN DELIVERY SYSTEM

8.1. *In situ* Nasal Gel

In the past, the administration of oral anti-Parkinson's drugs was always coupled with the intravenous route [94]. However, this route lacks target specificity resulting in the drug distribution throughout the body with limited brain distribution. This further leads to increased dose and dosing frequency resulting in severe adverse effects. In light of the-

se pitfalls associated with conventional therapy, the in administration route has attracted tremendous attention in systemic antiparkinsonian therapy [95]. Notably, intranasal therapy supports easy avoidance of the first-pass metabolism, increased blood passage, increased bioavailability, along with increased brain uptake of the drug. Additionally, this route is not only favored for drugs but also for peptides and oligonucleotides since it prevents degradation through enzymatic action through the intravenous route in the systemic circulation [96]. *In situ* gel-based anti-Parkinsonian therapeutics have emerged as a promising approach nowadays. The utmost noteworthy characteristic feature of *in situ* gels is the site-specific conversion from sol to gel phase. This promotes the formation of the depot at the desired site for sustained release, and prevents mucociliary clearance and local enzymatic degradation [97]. They primarily manifest fluid-like properties during administration but undergo conversion from sol to gel in response to numerous stimuli such as changes in ionic concentration, fluctuations in body temperature, changes in pH, or some other diversified changes in the biological environment [98-100]. *In situ* gel-based anti-Parkinson formulations (as mentioned in Table **3**) are widely accepted because of their versatility and various advantages such as enhanced bioavailability, improved patient acceptance, diminished interference with the biological environment, controlled release, and biodegradability.

The cold method is the most simplified method, which favors the formulation of the thermo- reversible form of *in situ* gels. The most pivotal step of this method comprises polymer solvation at an optimum temperature of 30^{o}C with a magnetic stirrer. The other primary ingredients to be added in the resulting formulation includes sodium chloride, polyethylene glycol, sodium chlorate, benzalkonium chloride ensured by the maintenance of an optimum temperature of 4^{o}C for consecutively 4 h. Henceforth, the final step includes the addition of an antiparkinsonian drug with continuous shaking at 100 pm followed by probe sonication. Recent studies included that Lungare *et al.* had prepared a thermoreversible *in situ* gel comprising amantadine (antiparkinsonian drug). In this formulation, they employed Pluronic F127, which is commonly known as Poloxamer 407, having notable thermo-reversible properties along with carboxymethyl cellulose with the marked mucoadhesive property. They observed three-stage gelation phenomena at 34 ± 1^{o}C and reproducible non-Newtonian behavior with good storage stability up to 8 weeks. The optimized intranasal formulation demonstrated no significant cytotoxicity to human nasal epithelia up to 4 mg/mL. With the utilization of a nasal cast model, deposition into the olfactory regions (potential nose-to-brain) was demonstrated on nozzle insertion (5 mm), whereas tilting of the head in the forward direction resulted in a greater deposition in the nasal cavity. Furthermore, the bioavailability and drug release profile of amantadine have increased to a greater extent compared to its oral-based counterpart [101]. Another similar approach by Rao *et al.* comprised ropinirole loaded onto thermo-reversible *in situ* gels whose matrix consisted of Poloxamer 407. Nasal *in situ* gel augmented 8.5 fold greater brain uptake compared to the intravenous route [75, 101-105]. Hoban *et al.* explored the delivery of neurotrophic factors across the brain through genetically altered mesenchymal stem cells for the management of neurodegenerative diseases. Poor survival post-transplantation followed by microglial stimulation and astrocyte employment at the graft site was observed. An *in-situ* gelation type I collagen hydrogel was investigated for intracerebral transplantation matrix for delivery of glia-derived neurotrophic factor (GDNF) overexpressing stem cells to the rat brain. *In vitro* characterization showed high biocompatibility and promoted GDNF secretion into the striatal parenchyma. Additionally, they demonstrated the transplantation of stem cells in a collagen hydrogel matrix significantly reduced the host brain's response by diminishing microglia and astrocyte stimulation at the bioactive site ($p < 0.01$). This approach displayed promising results and opened up new horizons in cell support and graft integration [106].

8.2. Cell-Penetrating Peptides

Cell-penetrating peptides comprise short-chain peptides which are cationic and amphipathic with inherent cell membrane traversing activity. Two types of CPP exist which include antimicrobial sequences and chimeric peptides. Binding of cationic CPP selectively to the brain endothelial membrane *via* electrostatic interaction. Kamei and co-workers envisaged the delivery of insulin to the brain parenchyma mediated by enhanced drug uptake across the nasal epithelium through neuronal cells to improve the potential of the N2B delivery. Insulin was intra-nasally co-administered with penetration, a cell-penetrating peptide to the mice. Intranasal administration promoted the penetration of distal regions of the brain through the nasal cavity with greater accumulation in the cerebral cortex, cerebellum, and brain stem. D-penetratin could help in insulin traversion across the brain with minimal risk of systemic exposure indicating the tremendous potential of cell-penetrating peptides [96]. Similarly, Khafagy and co-workers attempted to promote leptin delivery to the brain *via* intranasal co-administration with penetratin. *In vivo* studies revealed that leptin co-administered with L-penetratin was effectively absorbed and localized in the anterior section of the brain. Chronic leptin delivery *via* repeated intranasal co-administration with CPP diminished the appetite and reduced the bodyweight of rats with lower plasma triglyceride levels. Brain sample analysis indicated the phosphorylation of Stat3 *via* leptin receptor resulted in the therapeutic efficacy of leptin [107].

8.3. Intranasal Delivery of Genes Encoding Neurotrophic Factors for PD Treatment

The intranasal gene delivery system appears to be the most promising approach for the treatment of PD over recent years. It is almost impossible to deliver major and comparatively bigger biomolecules to the brain by crossing the BBB. The intranasal mode of drug delivery provides the facility of delivering neuroprotective biomolecules into the brain in a non-surgical way without involving passage *via* BBB. There is a possibility of delivering a gene of interest containing a vector. There are two types of vectors that can be used for delivering the gene and the selection of vector depends on which would have more transfection efficiency on IN delivery. *First*, one is a viral vector like a replication-defective adenoviral vector AdRSVfbgal, herpes simplex virus type1 (HSV-1), R830, Adeno associated virus (AAV) vector. *The second* is a non-viral vector that makes plasmid DNA construct and has lesser immunogenic efficiency. Latter includes plasmid DNA, PCMVβ which was detected in the brain after 10 min of IN

delivery. The other in this category is 10000 Da PEG substituted lysine 30-mernanoparticles (NPS), pUGG encoding fusion protein of plasmid Glia Derived Neurotrophic Factor (pGDNF) linked with a green fluorescent protein (GFP) [108]. Both viral and nonviral vectors also offer the potential for neurotrophic factor delivery. The major neuroprotective biomolecules delivered *via* the intranasal gene delivery system involve Neuropeptide Y and substance P, (GDNF), neurturin, *etc.* [109-112]. Besides neuropeptides, there are other peptide molecules such as the Basic fibroblast growth factor, transforming growth factor (TGF-β), Pituitary adenyl cyclase-activating peptide, cyclosporin-A, TNF- inhibitory single-chain antibody fragment ESBA105 have been tried at the preclinical level in PD by employing the IN delivery approach [113].

8.3.1. Neuropeptide

Neuropeptide Y is mainly a neuroprotective peptide widely expressed in the mammalian brain and consists of a 36 amino acids chain. It functions as a co-transmitter, which is released from post-ganglionic sympathetic neurons. Delivery of this peptide *via* intranasal way provides a promising betterment of treatment for Parkinson's disease. It also plays a major role in protecting the SH-SY5Y cell lines and dopaminergic cell death both in mice and rats from 6-hydroxydopamine- (6-OHDA)-induced toxicity [114]. Most recently, the intranasal delivery of later has alleviated the Machado-Joseph disease (MJD), a form of inherited ataxia [115]. In delivery of NPY for major depression, panic disorder, and social anxiety disorder is under clinical trial [116] and have proven effective for major depressive disorder patient in a randomized controlled trial [117]. NPY gene therapy employing a recombinant adeno-associated viral (rAAV) vector expressing the human NPY gene, has decreased the chronic spontaneous seizures in the rat [118]. Meanwhile, the effectiveness of IN gene delivery of NPY in PD further needs to be explored at the preclinical and clinical levels. Neuropeptide Substance P (SP) belongs to tachykinin peptide and is involved in the regulation of many biological processes. SP mediates neuro-immunomodulatory activities and neurogenic inflammation within the CNS and in the peripheral nervous system [119]. The in delivery of lipid-based SP-loaded gelatine nanoparticles (SP-GNP) has protected the PD-like behavior in (6-OHDA) induced hemiparkinsonian rats [120]. Although SP itself acts as the tool for specific delivery of various types of biomolecular cargo and has been tried in the case of targeting glioma but their gene delivery in the case of PD is still not reported [121, 122].

8.3.2. GDNF

GDNF & Neuturin have the potential for developing as PD modifying therapy as they can rescue damaged DA neurons by promoting their growth and hence will increase the DA production. The preclinical efficacy of GDNF did not get replicated at the clinical level in PD patients. The in delivery of GDNF and its liposome formulation has shown effectiveness in 6-OHDA induced PD model of rats [123]. Its gene delivery mainly involves the use of viral vectors like Adenovirus (Ad), Lentivirus (LV), or AAV for therapeutic importance encoding GDNF directly into the brain. Viral vectors like LV and AAV are randomly used because of their low immunogenic properties, safety margin, and increased transgenic properties. It has been reported in recent studies that Ad-GDNF, LV-GDNF have shown a greater potential for neuroprotection coupled with restorative effects of dopamine in experimental animal models [124-126]. Other studies in the line have delivered the plasmid DNA nanoparticles (NPs) encoding human GDNF using in route to rats which induces transgene expression in the brain and has protected the DA neurons in a 6-OHDA induced PD model of rats.

8.3.3. Neurturin

It belongs to one of the neuroprotective members of GDNF's family. It possesses the same potential and efficacy of neuroprotection and restorative effect the same as that of GDNF when delivered intranasally, as reported in both *in-vitro* and *in-vivo* experiments in animal models. The repeated intrastriatal gene delivery of AAV2-neurturin (CERE120) to advanced PD patient AAV2-neurturin (CERE120) has failed to alleviate the significant PD pathology due to neurturin expression [108, 126]. The physicochemical property of nurturing limits its use in PD, and hence the in delivery can be tried in the future.

8.4. Intranasal Delivery of Mitochondria

In recent times, N2B delivery of mitochondria, a nano-sized organelle has also been tried in many diseases' context [127]. Latter also lacks the HLA-Class 1 antigens and hence possesses less immunogenicity potential. Intranasally delivered mitochondria rapidly reach meninges where it gets internalized with the help of macrophages and revers the various neural deficits. Delivery of mitochondria has alleviated the cognitive defects in the oxaliplatin-induced chemotherapy model of rat where mitochondria isolated from human mesenchymal stem cell was delivered to mice *via* in route [128]. Similarly, in PD also in delivery of mitochondria has been tried recently, where 200 μg of allogenic mitochondrial isolated from the rat liver was conjugated with cell-penetrating peptide (Pep-1) and delivered once a week for 3 months to the 6-OHDA induced PD model of rats. Later, they have shown benefit in both rotational and locomotor behaviours and 60 % recovery in DA neurons [129] (Fig. **6**).

8.5. Focused Ultrasound Enhanced in Delivery

The current treatments of PD give only symptomatic relief and are unable to control the progression of the disease. Another approach, the Focused ultrasound technology (IN+FUS) (Fig. **7**), emerged as a novel, non-invasive, and safe IN delivery method for PD, which administers pharmacological agents directly to the brain through the olfactory nerve pathway [130, 131]. Due to specific brain targeting, it allows uniform drug distribution in site-specific areas compared to N2B delivery alone. FUS can treat PD with less risk of complications, and it could provide a non-invasive alternative to existing surgical treatment at a lower cost. When FUS-mediated BBB opening is coupled with IN delivery of molecules, it led to precise and accurate delivery of intranasally administered molecules into the brain without damaging surrounding normal tissue. Before FUS-induced BBB opening, microbubbles injected systemically begin to cavitate, and this microbubble cavitation in the targeted region of the FUS beam path enhances the delivery efficiency. Focused ultrasound enhanced in delivery study of BDNF in the PD mouse model was recently performed where the BDNF

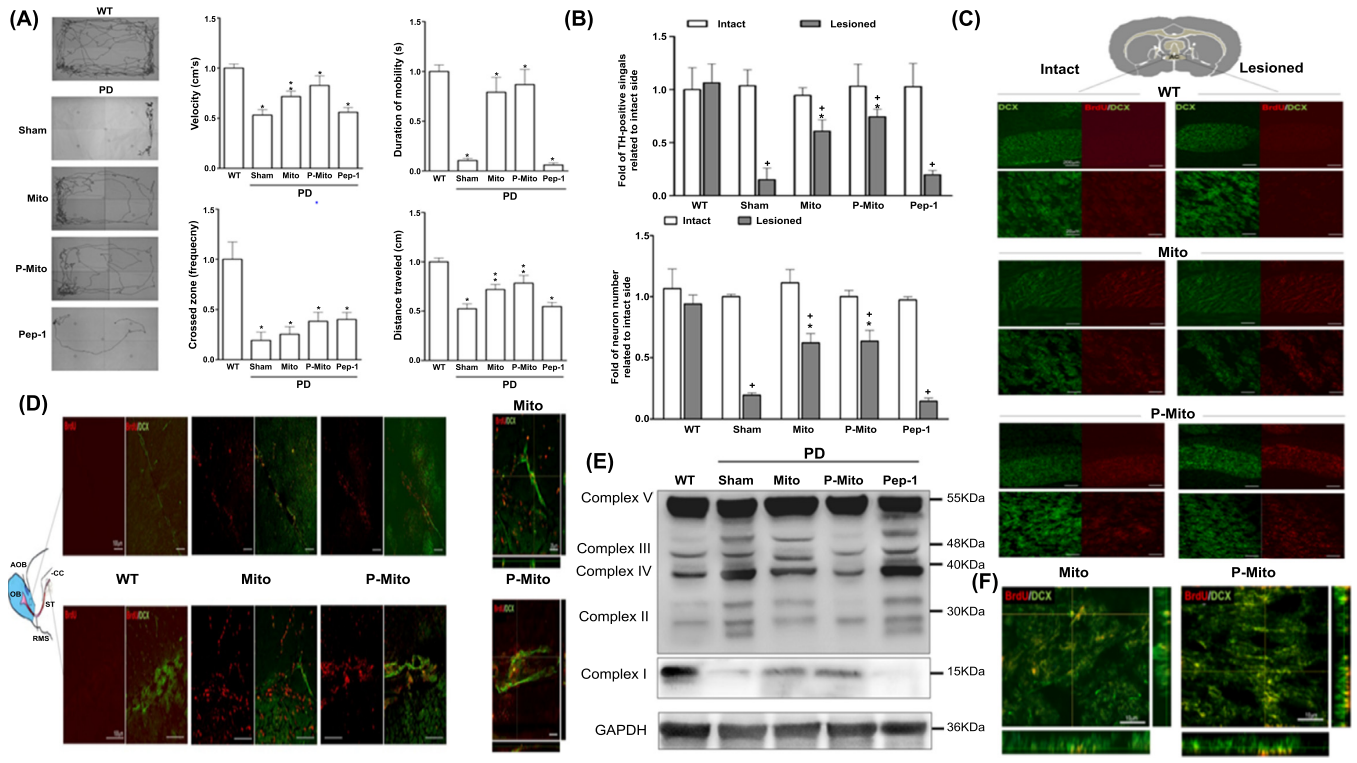

Fig. (6). (**A**) Intranasal treatment with allogeneic mitochondria ameliorates behaviour in Parkinson's disease rats as shown from the rotational response, open field test, zone crossing frequency and mobility, (**B**) Comparison of Nissl's positive neurons and TH positive signals in intact and lesioned cells, (**D**) Double immuno-fluorescent staining with doublecortin and BrdU antibodies demonstrating the uptake of exogenous mitochondria in DCX-positive migrating neurons, (**E**) Normalized mitochondrial complex expression in lesioned substantia nigra to display a reduction in oxidative stress, (**C**, **F**) Contralateral mitochondrial delivery with double immune-fluorescent staining, through BrdU (red) with DCX antibody (green) along with merged Z-stack confocal microscopy image for wild type, lesioned, mitochondrial and Pep-1 mitochondria. Reprinted from Chang *et al*. [129]. Copyright © 2021 licensed under CC BY 4.0 (http://creativecommons.org/licenses/by/4.0/). Published by Springer Nature. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

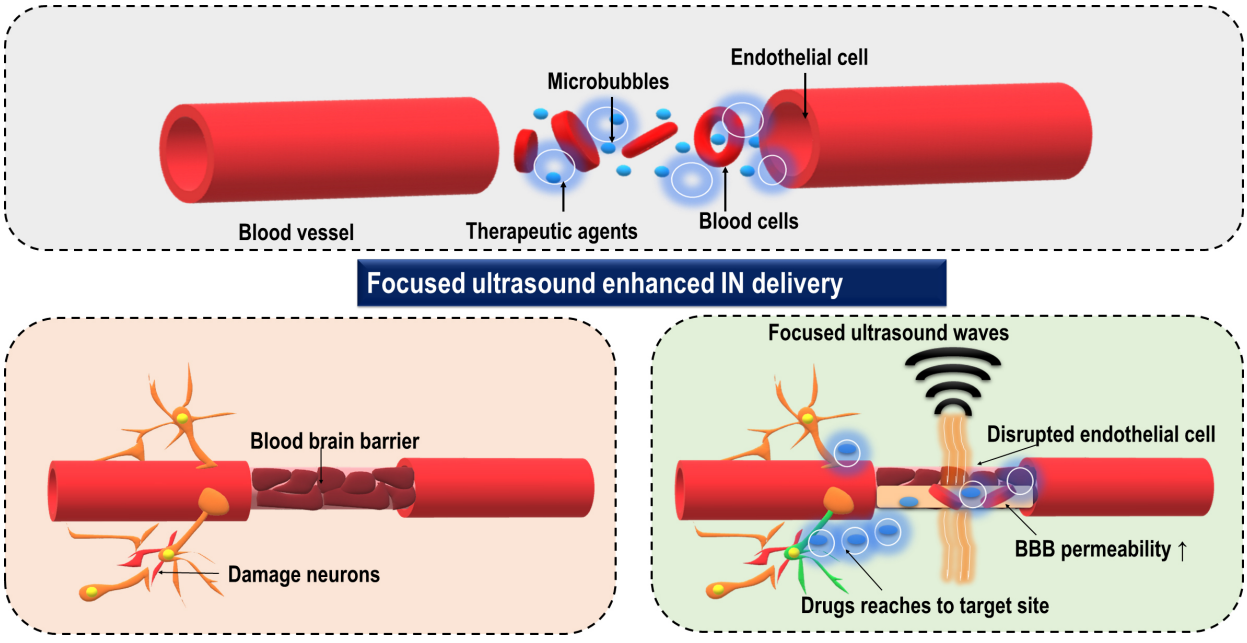

Fig. (7). An overview of focused ultrasound enhanced intra-nasal drug delivery. The focused ultrasound waves coupled with microbubbles enhance BBB permeability and deliver the drug to the target site. *(A higher resolution/colour version of this figure is available in the electronic copy of the article).*

by in route has improved the delivery in the targeted brain region by using FUS-induced BBB opening. Usually, in PD-associated DA neurons, dysfunction can lead to a decrease in the expression of tyrosine hydroxylase (TH), a marker of healthy DA neurons compared to normal mice. The BDNF was reported as a pharmacological agent that inhibits DA neuronal degeneration and stimulates DA synthesis in PD affected region [131]. It has led to the focused ultrasound enhanced N2B delivery as a novel potential approach for PD therapy.

CONCLUSION

The passage of the currently available anti-Parkinson drugs to the brain is restricted because BBB presents an unavoidable hurdle that limits the accessibility of drug therapy. Moreover, its complex anatomy restricts the evolution of new therapeutic approaches for treating PD. To improve significant brain targeting, recent studies and scientific experiments from the last few years scaffold the N2B drug delivery system as a novel approach for direct brain targeting. The nasal cavity has direct associations with olfactory and trigeminal nerves linked with nasal mucosa and the brain. The advantage of selecting the in-drug delivery system over currently available anti-Parkinson's therapy ensures more availability of the anti-Parkinson drugs to the target areas of the brain. Meanwhile, the N2B delivery also has its share of limitations, such as mucociliary clearance, drug degradation, translocation of drugs from the nasal cavity by efflux mechanisms, and less residence time in the nasal mucosa. Additionally, the collaboration of nanotechnology with the in approach to delivering drugs has diversified PD therapy to a greater extent. The combination of NP in in approach favours the drug delivery by increasing the BBB permeability, increased bioavailability, more patient acceptance, less interference with the biological environment, biodegradability, along with more site-specific delivery in the brain. The probable size required for NP for excellent delivery of anti-Parkinson drugs should be between 100 to 200 nm. As per the depiction in the review, studies also demonstrated that suitable surface-modified nano-carriers such as chitosan NP, PLGA NP, nanoemulsion, solid-lipid NP, lipid NP were used for enhancing the brain-specific targeting prospective of anti-PD therapy. Similarly, recent advances (for rapid brain uptake) in the N2B delivery for PD comprise the usage of In-situ gels loaded with anti-Parkinson drugs, inhaled levodopa therapy, focused ultrasound enhancing in delivery along with intra-nasal gene therapy (delivery of big sized neuroprotective biomolecules directly into the brain) including direct mitochondria delivery. Hence, it can be concluded that recent development in in approach of drug delivery coupled with nanotechnology has opened a new horizon for PD treatment, ensuring more availability and more accuracy. However, more studies are required to evaluate the efficacy of therapy *via* N2B delivery as enough research is not available. To bring the therapy to clinical premises strong research background in terms of formulation development and assessment of their optimal delivery to the brain.

LIST OF ABBREVIATIONS

PD = Parkinson's Disease

NDD = Neurodegenerative Disorder

DA = Dopamine

AD = Alzheimer's Disease

MPTP = 1, 2, 3, 6-Methyl-Phenyl-Tetrahydropyridine

PTEN = Phosphatase, and Tensin Homolog

CONSENT FOR PUBLICATION

Not applicable.

FUNDING

None.

CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

ACKNOWLEDGEMENTS

The authors would like to acknowledge the Department of Pharmaceuticals, Ministry of Chemicals and Fertilizers, Government of India for their financial support.

REFERENCES

[1] Simon, D.K.; Tanner, C.M.; Brundin, P. Parkinson disease epidemiology, pathology, genetics, and pathophysiology. *Clin. Geriatr. Med.,* **2020**, *36*(1), 1-12. http://dx.doi.org/10.1016/j.cger.2019.08.002 PMID: 31733690

[2] Maiti, P.; Manna, J.; Dunbar, G.L.; Maiti, P.; Dunbar, G.L. Current understanding of the molecular mechanisms in Parkinson's disease: Targets for potential treatments. *Transl. Neurodegener.,* **2017**, *6*(1), 28. http://dx.doi.org/10.1186/s40035-017-0099-z PMID: 29090092

[3] Gabathuler, R. Approaches to transport therapeutic drugs across the blood-brain barrier to treat brain diseases. *Neurobiol. Dis.,* **2010**, *37*(1), 48-57. http://dx.doi.org/10.1016/j.nbd.2009.07.028 PMID: 19664710

[4] Kaur, R.; Mehan, S.; Singh, S. Understanding multifactorial architecture of Parkinson's disease: Pathophysiology to management. In: *Neurological Sciences*; Springer: Verlag Italia, **2019**; 40, pp. 13-23.

[5] Pajouhesh, H.; Lenz, G.R. Medicinal chemical properties of successful central nervous system drugs. *NeuroRx,* **2005**, *2*(4), 541-553. http://dx.doi.org/10.1602/neurorx.2.4.541 PMID: 16489364

[6] Engelhardt, B.; Sorokin, L. The blood-brain and the blood-cerebrospinal fluid barriers: Function and dysfunction. *Semin. Immunopathol.,* **2009**, *31*(4), 497-511. http://dx.doi.org/10.1007/s00281-009-0177-0 PMID: 19779720

[7] Silindir, G.M.; Yekta, O.A.; Chalon, S. Drug delivery systems for imaging and therapy of Parkinson's disease. *Curr. Neuropharmacol.,* **2016**, *14*(4), 376-391. http://dx.doi.org/10.2174/1570159X14666151230124904 PMID: 26714584

[8] Djupesland, P.G.; Messina, J.C.; Mahmoud, R.A. The nasal approach to delivering treatment for brain diseases: An anatomic, physiologic, and delivery technology overview. *Ther. Deliv.,* **2014**, *5*(6), 709-733. http://dx.doi.org/10.4155/tde.14.41 PMID: 25090283

[9] Haddad, F.; Sawalha, M.; Khawaja, Y.; Najjar, A.; Karaman, R. Dopamine and levodopa prodrugs for the treatment of Parkinson's disease. *Molecules,* **2017**, *23*(1), 40. http://dx.doi.org/10.3390/molecules23010040 PMID: 29295587

[10] Tambasco, N.; Romoli, M.; Calabresi, P. Levodopa in Parkinson's disease: Current status and future developments. *Curr. Neuropharmacol.,* **2018**, *16*(8), 1239-1252.

http://dx.doi.org/10.2174/1570159X15666170510143821 PMID: 28494719

[11] Joseph, J. Motor fluctuations and dyskinesias in Parkinson's disease: Clinical manifestations. *Mov Disord.,* **2005**, *20*(Suppl 11), S11-S16.

[12] Angela, M.C. Presynaptic mechanisms of l-DOPA-induced dyskinesia: The findings, the debate, and the therapeutic implications. *Front. Neurol.,* **2014**, 242, 5.

[13] Olarte-Avellaneda, S.; Cepeda Del Castillo, J.; Rojas-Rodriguez, A.F.; Sánchez, O.; Rodríguez-López, A.; Suárez, G.D.A.; Pulido, L.M.S.; Almériga-Díaz, C.J. Bromocriptine as a novel pharmacological chaperone for mucopolysaccharidosis IV A. *ACS Med. Chem. Lett.,* **2020**, *11*(7), 1377-1385. http://dx.doi.org/10.1021/acsmedchemlett.0c00042 PMID: 32676143

[14] Brooks, D.J. Dopamine agonists: Their role in the treatment of Parkinson's disease. *J. Neurol. Neurosurg. Psychiatry,* **2000**, *68*(6), 685-689. http://dx.doi.org/10.1136/jnnp.68.6.685 PMID: 10811688

[15] Olanow, C.W.; Kieburtz, K.; Leinonen, M.; Elmer, L.; Giladi, N.; Hauser, R.A.; Klepiskaya, O.S.; Kreitzman, D.L.; Lew, M.F.; Russell, D.S.; Kadosh, S.; Litman, P.; Friedman, H.; Linvah, N. The PB Study Group, A randomized trial of a low-dose Rasagiline and Pramipexole combination (P2B001) in early Parkinson's disease. *Mov. Disord.,* **2017**, *32*(5), 783-789. http://dx.doi.org/10.1002/mds.26941 PMID: 28370340

[16] Antonini, A; Abbruzzese, G; Barone, P; Bonuccelli, U; Lopiano, L.; Onofrj, M. COMT inhibition with tolcapone in the treatment algorithm of patients with Parkinson's disease (PD): Relevance for motor and non-motor features. *Neuropsychiatr Dis Treat,* **2008**, *4*(1 A), 1-9.

[17] Onofrj, M.; Thomas, A.; Iacono, D.; Di Iorio, A.; Bonanni, L. Switch-over from tolcapone to entacapone in severe Parkinson's disease patients. *Eur. Neurol.,* **2001**, *46*(1), 11-16. http://dx.doi.org/10.1159/000050749 PMID: 11455177

[18] Parkinson Study Group. Entacapone improves motor fluctuations in levodopa- treated Parkinson's disease patients. *Ann. Neurol.,* **1997**, *42*(5), 747-755. http://dx.doi.org/10.1002/ana.410420511 PMID: 9392574

[19] Gordin, A.; Kaakkola, S.; Teräväinen, H. Clinical advantages of COMT inhibition with entacapone - a review. *J. Neural Transm. (Vienna),* **2004**, *111*(10-11), 1343-1363. http://dx.doi.org/10.1007/s00702-004-0190-3 PMID: 15340869

[20] Riederer, P.; Lachenmayer, L.; Laux, G. Clinical applications of MAO-inhibitors. *Curr. Med. Chem.,* **2004**, *11*(15), 2033-2043. http://dx.doi.org/10.2174/0929867043364775 PMID: 15279566

[21] Schapira, A.H. Monoamine oxidase B inhibitors for the treatment of Parkinson's disease: A review of symptomatic and potential disease-modifying effects. *CNS Drugs,* **2011**, *25*(12), 1061-1071. http://dx.doi.org/10.2165/11596310-000000000-00000 PMID: 22133327

[22] Krishna, R.; Ali, M.; Moustafa, A.A. Effects of combined MAO-B inhibitors and levodopa *vs.* monotherapy in Parkinson's disease. *Front. Aging Neurosci.,* **2014**, *6*(JUL), 180. PMID: 25120478

[23] Schwab, R.S.; England, A.C., Jr; Poskanzer, D.C.; Young, R.R. Amantadine in the treatment of Parkinson's disease. *JAMA,* **1969**, *208*(7), 1168-1170. http://dx.doi.org/10.1001/jama.1969.03160070046011 PMID: 5818715

[24] Malkani, R.; Zadikoff, C.; Melen, O.; Videnovic, A.; Borushko, E.; Simuni, T. Amantadine for freezing of gait in patients with Parkinson disease. *Clin. Neuropharmacol.,* **2012**, *35*(6), 266-268. http://dx.doi.org/10.1097/WNF.0b013e31826e3406 PMID: 23123688

[25] Müller, T. Drug therapy in patients with Parkinson's disease. *Transl. Neurodegener.,* **2012**, *1*(1), 10. http://dx.doi.org/10.1186/2047-9158-1-10 PMID: 23211041

[26] Current pharmacotherapy and screening models for Parkinson's disease- a mini review. *J. Nat. Remed.,* Available from: https://www.jnronline.com/ojs/index.php/about/article/view/192 [Accessed on: 2022 Jun, 1].

[27] Daniele, A.; Albanese, A.; Gainotti, G.; Gregori, B.; Bartolomeo, P. Zolpidem in Parkinson's disease. *Lancet,* **1997**, *349*(9060), 1222-1223. http://dx.doi.org/10.1016/S0140-6736(05)62416-6

[28] Hajak, G.; Müller, W.E.; Wittchen, H.U.; Pittrow, D.; Kirch, W. Abuse and dependence potential for the non-benzodiazepine hypnotics zolpidem and zopiclone: A review of case reports and epidemiological data. *Addiction,* **2003**, *98*(10), 1371-1378. http://dx.doi.org/10.1046/j.1360-0443.2003.00491.x PMID: 14519173

[29] Bronstein, J.M.; Tagliati, M.; Alterman, R.L.; Lozano, A.M.; Volkmann, J.; Stefani, A.; Horak, F.B.; Okun, M.S.; Foote, K.D.; Krack, P.; Pahwa, R.; Henderson, J.M.; Hariz, M.I.; Bakay, R.A.; Rezai, A.; Marks, W.J., Jr; Moro, E.; Vitek, J.L.; Weaver, F.M.; Gross, R.E.; DeLong, M.R. Deep brain stimulation for Parkinson disease: An expert consensus and review of key issues. *Arch. Neurol.,* **2011**, *68*(2), 165-171. http://dx.doi.org/10.1001/archneurol.2010.260 PMID: 20937936

[30] deSouza, R.M.; Moro, E.; Lang, A.E.; Schapira, A.H.V. Timing of deep brain stimulation in Parkinson disease: A need for reappraisal? *Ann. Neurol.,* **2013**, *73*(5), 565-575. http://dx.doi.org/10.1002/ana.23890 PMID: 23483564

[31] Deuschl, G.; Paschen, S.; Witt, K. Clinical outcome of deep brain stimulation for Parkinson's disease. *Handb. Clin. Neurol.,* **2013**, *116*(part II), 107-128. http://dx.doi.org/10.1016/B978-0-444-53497-2.00010-3 PMID: 24112889

[32] Tyulmankov, D.; Tass, P.A.; Bokil, H. Periodic flashing coordinated reset stimulation paradigm reduces sensitivity to ON and OFF period durations. *PLoS One,* **2018**, *13*(9), e0203782. http://dx.doi.org/10.1371/journal.pone.0203782 PMID: 30192855

[33] Lenz, F.A. Ablative surgery for the treatment of Parkinson's disease. *Handbook of Clinical Neurology*; Elsevier, **2007**, pp. 243-260.

[34] Lang, A.E.; Obeso, J.A. Challenges in Parkinson's disease: Restoration of the nigrostriatal dopamine system is not enough. *Lancet Neurol.,* **2004**, *3*(5), 309-316. http://dx.doi.org/10.1016/S1474-4422(04)00740-9 PMID: 15099546

[35] Rivetti di Val Cervo, P.; Romanov, R.A.; Spigolon, G.; Masini, D.; Martín-Montañez, E.; Toledo, E.M. Induction of functional dopamine neurons from human astrocytes *in vitro* and mouse astrocytes in a Parkinson's disease model. *Nat. Biotechnol.,* **2017**, *35*(5), 444-452. http://dx.doi.org/10.1038/nbt.3835

[36] Goodarzi, P.; Aghayan, H.R.; Larijani, B.; Soleimani, M.; Dehpour, A.R.; Sahebjam, M.; Ghaderi, F.; Arjmand, B. Stem cell-based approach for the treatment of Parkinson's disease. *Med. J. Islam. Repub. Iran,* **2015**, *29*, 168. PMID: 26000262

[37] Kuhn, W; Müller, T. Medications for treating Parkinson's disease. 8.

[38] Perez-Lloret, S.; Barrantes, F.J. Deficits in cholinergic neurotransmission and their clinical correlates in Parkinson's disease. *NPJ Park. Dis.,* **2016**, *2*(1), 1-12.

[39] Daniele, A.; Panza, F.; Greco, A.; Logroscino, G.; Seripa, D. Can a positive allosteric modulation of GABAergic receptors improve motor symptoms in patients with Parkinson's disease? The potential role of zolpidem in the treatment of Parkinson's disease. *Parkinsons Dis.,* **2016**, *2016.* http://dx.doi.org/10.1155/2016/2531812

[40] Lee, D.; Dallapiazza, R.; De Vloo, P.; Lozano, A. Current surgical treatments for Parkinson's disease and potential therapeutic targets. *Neural Regen. Res.,* **2018**, *13*(8), 1342-1345. http://dx.doi.org/10.4103/1673-5374.235220 PMID: 30106037

[41] Md, S.; Bhattmisra, S.K.; Zeeshan, F.; Shahzad, N.; Mujtaba, M.A.; Srikanth Meka, V.; Radhakrishnan, A.; Kesharwani, P.; Baboota, S.; Ali, J. Nano-carrier enabled drug delivery systems for nose to brain targeting for the treatment of neurodegenerative disorders. *J. Drug Deliv. Sci. Technol.,* **2018**, *43*, 295-310. http://dx.doi.org/10.1016/j.jddst.2017.09.022

[42] Hussein, N.R.; Omer, H.K.; Elhissi, A.M.A.; Ahmed, W. Advances in nasal drug delivery systems. In: *Advances in Medical and Surgical Engineering*; Elsevier Inc., **2020**; pp. 279-311. http://dx.doi.org/10.1016/B978-0-12-819712-7.00015-2

[43] Hirlekar, R.S.; Momin, A.M. Advances in drug delivery from nose to brain: An overview. *Curr. Drug Ther.,* **2018**, *13*(1), 4-24. http://dx.doi.org/10.2174/1574885512666170921145204

[44] Selvaraj, K.; Gowthamarajan, K.; Karri, V.V.S.R. Nose to brain transport pathways an overview: Potential of nanostructured lipid carriers in nose to brain targeting. *Artif. Cells Nanomed. Biotechnol.,* **2018**, *46*(8), 2088-2095. PMID: 29282995

[45] Semwal, R.; Upadhyaya, K.; Semwal, R.B.; Semwal, D.K. Acceptability of nose-to-brain drug targeting in context to its advances and challenges. *Drug Deliv. Lett.,* **2017**, *8*(1), 20-28.

[46] Bourganis, V.; Kammona, O.; Alexopoulos, A.; Kiparissides, C. Recent advances in carrier mediated nose-to-brain delivery of pharmaceutics. *Eur. J. Pharm. Biopharm.,* **2018**, *128*, 337-362. http://dx.doi.org/10.1016/j.ejpb.2018.05.009 PMID: 29733950

[47] Alexander, A.; Agrawal, M.; Bhupal Chougule, M.; Saraf, S.; Saraf, S. Nose-to-brain drug delivery: An alternative approach for effective brain drug targeting. an alternative approach for effective brain drug targeting. In: *Nanopharmaceuticals: Volume 1: Expectations and Realities of Multifunctional Drug Delivery Systems*; Elsevier Inc., **2020**; pp. 175-200.

[48] Ross, T.M.; Martinez, P.M.; Renner, J.C.; Thorne, R.G.; Hanson, L.R.; Frey, W.H., II Intranasal administration of interferon beta bypasses the blood-brain barrier to target the central nervous system and cervical lymph nodes: A non-invasive treatment strategy for multiple sclerosis. *J. Neuroimmunol.,* **2004**, *151*(1-2), 66-77. http://dx.doi.org/10.1016/j.jneuroim.2004.02.011 PMID: 15145605

[49] Thorne, R.G.; Pronk, G.J.; Padmanabhan, V.; Frey, W.H., II 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*(2), 481-496. http://dx.doi.org/10.1016/j.neuroscience.2004.05.029 PMID: 15262337

[50] Ghadiri, M.; Young, P.; Traini, D. Strategies to enhance drug absorption *via* nasal and pulmonary routes. *Pharmaceutics,* **2019**, *11*(3), 113. http://dx.doi.org/10.3390/pharmaceutics11030113 PMID: 30861990

[51] Keller, L-A.; Merkel, O.; Popp, A. Intranasal drug delivery: Opportunities and toxicologic challenges during drug development. *Drug Deliv. Transl. Res.,* **2021**, *2021*, 1-23. PMID: 33491126

[52] Iliff, J.J.; Wang, M.; Liao, Y.; Plogg, B.A.; Peng, W.; Gundersen, G.A.; Benveniste, H.; Vates, G.E.; Deane, R.; Goldman, S.A.; Nagelhus, E.A.; Nedergaard, M. A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. *Sci. Transl. Med.,* **2012**, *4*(147), 147ra111. http://dx.doi.org/10.1126/scitranslmed.3003748 PMID: 22896675

[53] Aspelund, A.; Antila, S.; Proulx, S.T.; Karlsen, T.V.; Karaman, S.; Detmar, M.; Wiig, H.; Alitalo, K. A dural lymphatic vascular system that drains brain interstitial fluid and macromolecules. *J. Exp. Med.,* **2015**, *212*(7), 991-999. http://dx.doi.org/10.1084/jem.20142290 PMID: 26077718

[54] Inoue, D.; Furubayashi, T.; Tanaka, A.; Sakane, T.; Sugano, K. Effect of cerebrospinal fluid circulation on nose-to-brain direct delivery and distribution of caffeine in rats. *Mol. Pharm.,* **2020**, *17*(11), 4067-4076. http://dx.doi.org/10.1021/acs.molpharmaceut.0c00495 PMID: 32955898

[55] Lilius, T.O.; Blomqvist, K.; Hauglund, N.L.; Liu, G.; Stæger, F.F.; Bærentzen, S.; Du, T.; Ahlström, F.; Backman, J.T.; Kalso, E.A.; Rauhala, P.V.; Nedergaard, M. Dexmedetomidine enhances glymphatic brain delivery of intrathecally administered drugs. *J. Control. Release,* **2019**, *304*, 29-38. http://dx.doi.org/10.1016/j.jconrel.2019.05.005 PMID: 31067483

[56] Marttin, E.; Schipper, N.G.M.; Verhoef, J.C.; Merkus, F.W.H.M. Nasal mucociliary clearance as a factor in nasal drug delivery. *Adv. Drug Deliv. Rev.,* **1998**, *29*(1-2), 13-38. http://dx.doi.org/10.1016/S0169-409X(97)00059-8 PMID: 10837578

[57] Anand, U; Feridooni, T; Agu, RU. Novel mucoadhesive polymers for nasal drug delivery. *Recent Adv. Novel Drug Carrier Syst.*, **2012**, 315-330. http://dx.doi.org/10.5772/52560

[58] Pathak, R.; Prasad Dash, R.; Misra, M.; Nivsarkar, M. Role of mucoadhesive polymers in enhancing delivery of nimodipine microemulsion to brain *via* intranasal route. *Acta Pharm. Sin. B,* **2014**, *4*(2), 151-160. http://dx.doi.org/10.1016/j.apsb.2014.02.002 PMID: 26579378

[59] Chari, S.; Sridhar, K.; Walenga, R.; Kleinstreuer, C. Computational analysis of a 3D mucociliary clearance model predicting nasal drug uptake. *J. Aerosol Sci.,* **2021**, *155*, 105757. http://dx.doi.org/10.1016/j.jaerosci.2021.105757

[60] van Rumund, A; Pavelka, L; Esselink, RAJ; Geurtz, BPM; Wevers, RA; Mollenhauer, B Peripheral decarboxylase inhibitors paradoxically induce aromatic L-amino acid decarboxylase. *NPJ Park Dis,* **2021**, *7*(1), 1-5.

[61] Cacciatore, I.; Ciulla, M.; Marinelli, L.; Eusepi, P.; Di Stefano, A. Advances in prodrug design for Parkinson's disease. *Expert Opin. Drug Discov.*, **2018**, *13*(4), 295-305. http://dx.doi.org/10.1080/17460441.2018.1429400 PMID: 29361853

[62] Bors, L.A.; Bajza, Á.; Mándoki, M.; Tasi, B.J.; Cserey, G.; Imre, T.; Szabó, P.; Erdő, F. Modulation of nose-to-brain delivery of a P-glycoprotein (MDR1) substrate model drug (quinidine) in rats. *Brain Res. Bull.,* **2020**, *160*, 65-73. http://dx.doi.org/10.1016/j.brainresbull.2020.04.012 PMID: 32344126

[63] Dolberg, A.M.; Reichl, S. Expression of P-glycoprotein in excised human nasal mucosa and optimized models of RPMI 2650 cells. *Int. J. Pharm.,* **2016**, *508*(1-2), 22-33. http://dx.doi.org/10.1016/j.ijpharm.2016.05.010 PMID: 27155589

[64] Di Gioia, S.; Trapani, A.; Mandracchia, D.; De Giglio, E.; Cometa, S.; Mangini, V.; Arnesano, F.; Belgiovine, G.; Castellani, S.; Pace, L.; Lavecchia, M.A.; Trapani, G.; Conese, M.; Puglisi, G.; Cassano, T. Intranasal delivery of dopamine to the striatum using glycol chitosan/sulfobutylether-β-cyclodextrin based nanoparticles. *Eur. J. Pharm. Biopharm.,* **2015**, *94*(1), 180-193. http://dx.doi.org/10.1016/j.ejpb.2015.05.019 PMID: 26032293

[65] Nagpal, K.; Singh, S.K.; Mishra, D.N. Chitosan nanoparticles: A promising system in novel drug delivery. *Chem. Pharm. Bull. (Tokyo),* **2010**, *58*(11), 1423-1430. http://dx.doi.org/10.1248/cpb.58.1423 PMID: 21048331

[66] Henriksen, I.; Green, K.L.; Smart, J.D.; Smistad, G.; Karlsen, J. Bioadhesion of hydrated chitosans: An *in vitro* and *in vivo* study. *Int. J. Pharm.,* **1996**, *145*(1-2), 231-240. http://dx.doi.org/10.1016/S0378-5173(96)04776-X

[67] Ozsoy, Y.; Gungor, S.; Cevher, E. Nasal delivery of high molecular weight drugs. *Molecules,* **2009**, *14*(9), 3754-3779. http://dx.doi.org/10.3390/molecules14093754 PMID: 19783956

[68] Raj, R.; Wairkar, S.; Sridhar, V.; Gaud, R. Pramipexole dihydrochloride loaded chitosan nanoparticles for nose to brain delivery: Development, characterization and *in vivo* anti-Parkinson activity. *Int. J. Biol. Macromol.,* **2018**, *109*, 27-35. http://dx.doi.org/10.1016/j.ijbiomac.2017.12.056 PMID: 29247729

[69] Nguyen, T.T.; Dung Nguyen, T.T.; Vo, T.K.; Tran, N.M.A.; Nguyen, M.K.; Van Vo, T.; Van Vo, G. Nanotechnology-based drug delivery for central nervous system disorders. *Biomed. Pharmacother.,* **2021**, *143*, 112117. http://dx.doi.org/10.1016/j.biopha.2021.112117 PMID: 34479020

[70] Mustafa, G.; Baboota, S.; Ahuja, A.; Ali, J. Formulation development of chitosan coated intra nasal ropinirole nanoemulsion for better management option of parkinson: An *in vitro ex vivo* evaluation. *Curr. Nanosci.,* **2012**, *8*(3), 348-360. http://dx.doi.org/10.2174/157341312800620331

[71] Bi, C.; Wang, A.; Chu, Y.; Liu, S.; Mu, H.; Liu, W.; Wu, Z.; Sun, K.; Li, Y. Intranasal delivery of rotigotine to the brain with lactoferrin-modified PEG-PLGA nanoparticles for Parkinson's disease treatment. *Int. J. Nanomed.,* **2016**, *11*, 6547-6559. http://dx.doi.org/10.2147/IJN.S120939 PMID: 27994458

[72] Sridhar, V.: Gaud, R.; Bajaj, A.; Wairkar, S. Pharmacokinetics and pharmacodynamics of intranasally administered selegiline nanoparticles with improved brain delivery in Parkinson's disease. *Nanomedicine (Lond.),* **2018**, *14*(8), 2609-2618.

[73] Ahmad, N. Rasagiline-encapsulated chitosan-coated PLGA nanoparticles targeted to the brain in the treatment of parkinson's disease. **2017**, *40*(13), 677-690. http://dx.doi.org/10.1080/10826076.2017.1343735

[74] Choudhury, H.; Zakaria, N.F.B.; Tilang, P.A.B.; Tzeyung, A.S.; Pandey, M.; Chatterjee, B.; Alhakamy, N.A.; Bhattamishra, S.K.; Kesharwani, P.; Gorain, B.; Md, S. Formulation development and evaluation of rotigotine mucoadhesive nanoemulsion for intranasal delivery. *J. Drug Deliv. Sci. Technol.,* **2019**, *54*, 101301. http://dx.doi.org/10.1016/j.jddst.2019.101301

[75] Gaba, B.; Khan, T.; Haider, M.F.; Alam, T.; Baboota, S.; Parvez, S.; Ali, J. Vitamin E loaded naringenin nanoemulsion *via* intranasal delivery for the management of oxidative stress in a 6-OHDA Parkinson's disease model. *Biomed. Res. Int.,* **2019**, *2019,* 2382563

[76] Pangeni, R.: Sharma, S.; Mustafa, G.; Ali, J.; Baboota, S.Vitamin E loaded resveratrol nanoemulsion for brain targeting for the treatment of Parkinson's disease by reducing oxidative stress. *Nanotechnology,* **2014**, *25*(48), 485102.

[77] Hernando, S.; Herran, E.; Figueiro-Silva, J.; Pedraz, J.L.; Igartua, M.; Carro, E.; Hernandez, R.M. Intranasal administration of TAT-conjugated lipid nanocarriers loading GDNF for Parkinson's disease. *Mol. Neurobiol.,* **2018**, *55*(1), 145-155. http://dx.doi.org/10.1007/s12035-017-0728-7 PMID: 28866799

[78] Mandal, S.; Das Mandal, S.; Chuttani, K.; Sawant, K.K.; Subudhi, B.B. Neuroprotective effect of ibuprofen by intranasal application of mucoadhesive nanoemulsion in MPTP induced Parkinson model. *J. Pharm. Investig,* **2015**, *46*(1), 41-53.

[79] Kumar, S.; Dang, S.; Nigam, K.; Ali, J.; Baboota, S. Selegiline nanoformulation in attenuation of oxidative stress and upregulation of dopamine in the brain for the treatment of Parkinson's disease. *Rejuvenation Res.,* **2018**, *21*(5), 464-476. http://dx.doi.org/10.1089/rej.2017.2035 PMID: 29717617

[80] Jafarieh, O.; Md, S.; Ali, M.; Baboota, S.; Sahni, J.K.; Kumari, B.; Bhatnagar, A.; Ali, J. Design, characterization, and evaluation of intranasal delivery of ropinirole-loaded mucoadhesive nanoparticles for brain targeting. *Drug Dev. Ind. Pharm.,* **2015**, *41*(10), 1674-1681. http://dx.doi.org/10.3109/03639045.2014.991400 PMID: 25496439

[81] Wang, F.; Yang, Z.; Liu, M.; Tao, Y.; Li, Z.; Wu, Z.; Gui, S. Facile nose-to-brain delivery of rotigotine-loaded polymer micelles thermosensitive hydrogels: *In vitro* characterization and *in vivo* behavior study. *Int. J. Pharm.,* **2020**, *577*, 119046. http://dx.doi.org/10.1016/j.ijpharm.2020.119046 PMID: 31982559

[82] Ahmad, E.; Lv, Y.; Zhu, Q.; Qi, J.; Dong, X.; Zhao, W.; Chen, Z.; Wu, W.; Lu, Y. TAT modification facilitates nose-to-brain transport of intact mPEG-PDLLA micelles: Evidence from aggregation-caused quenching probes. *Appl. Mater. Today,* **2020**, *19*, 100556. http://dx.doi.org/10.1016/j.apmt.2020.100556

[83] Migliore, M.M.; Ortiz, R.; Dye, S.; Campbell, R.B.; Amiji, M.M.; Waszczak, B.L. Neurotrophic and neuroprotective efficacy of intranasal GDNF in a rat model of Parkinson's disease. *Neuroscience,* **2014**, *274*, 11-23. http://dx.doi.org/10.1016/j.neuroscience.2014.05.019 PMID: 24845869

[84] Pardeshi, C.V.; Rajput, P.V.; Belgamwar, V.S.; Tekade, A.R.; Surana, S.J. Novel surface modified solid lipid nanoparticles as intranasal carriers for ropinirole hydrochloride: Application of factorial design approach. *Drug Deliv.,* **2013**, *20*(1), 47-56. http://dx.doi.org/10.3109/10717544.2012.752421 PMID: 23311653

[85] Kumar, M.; Pandey, R.S.; Patra, K.C.; Jain, S.K.; Soni, M.L.; Dangi, J.S.; Madan, J. Evaluation of neuropeptide loaded trimethyl chitosan nanoparticles for nose to brain delivery. *Int. J. Biol. Macromol.,* **2013**, *61*, 189-195. http://dx.doi.org/10.1016/j.ijbiomac.2013.06.041 PMID: 23831532

[86] Tan, J.P.K.; Voo, Z.X.; Lim, S.; Venkataraman, S.; Ng, K.M.; Gao, S.; Hedrick, J.L.; Yang, Y.Y. Effective encapsulation of apomorphine into biodegradable polymeric nanoparticles through a reversible chemical bond for delivery across the blood-brain barrier. *Nanomedicine,* **2019**, *17*, 236-245. http://dx.doi.org/10.1016/j.nano.2019.01.014 PMID: 30738234

[87] Liao, W.; Liu, Z.; Zhang, T.; Sun, S.; ye, J.; Li, Z.; Mao, L.; Ren, J. Enhancement of anti-inflammatory properties of nobiletin in macrophages by a nano-emulsion preparation. *J. Agric. Food Chem.,* **2018**, *66*(1), 91-98. http://dx.doi.org/10.1021/acs.jafc.7b03953 PMID: 29236498

[88] Solans, C.; Izquierdo, P.; Nolla, J.; Azemar, N.; Garcia-Celma, M.J. Nano-emulsions. *Curr. Opin. Colloid Interface Sci.,* **2005**, *10*(3-4), 102-110. http://dx.doi.org/10.1016/j.cocis.2005.06.004

[89] Ashhar, M.U.; Kumar, S.; Ali, J.; Baboota, S. CCRD based development of bromocriptine and glutathione nanoemulsion tailored ultrasonically for the combined anti-parkinson effect. *Chem. Phys. Lipids,* **2021**, *235*, 105035. http://dx.doi.org/10.1016/j.chemphyslip.2020.105035 PMID: 33400967

[90] Nehal, N.; Nabi, B.; Rehman, S.; Pathak, A.; Iqubal, A.; Khan, S.A.; Yar, M.S.; Parvez, S.; Baboota, S.; Ali, J. Chitosan coated synergistically engineered nanoemulsion of Ropinirole and nigella oil in the management of Parkinson's disease: Formulation perspective and *in vitro* and *in vivo* assessment. *Int. J. Biol. Macromol.,* **2021**, *167*, 605-619. http://dx.doi.org/10.1016/j.ijbiomac.2020.11.207 PMID: 33278450

[91] Hernando, S.; Gartziandia, O.; Herran, E.; Pedraz, J.L.; Igartua, M.; Hernandez, R.M. Advances in nanomedicine for the treatment of Alzheimer's and Parkinson's diseases. *Nanomedicine (Lond.),* **2016**, *11*(10), 1267-1285. http://dx.doi.org/10.2217/nnm-2016-0019 PMID: 27077453

[92] Sun, Y.; Li, L.; Xie, H.; Wang, Y.; Gao, S.; Zhang, L.; Bo, F.; Yang, S.; Feng, A. Primary studies on construction and evaluation of ion-sensitive *in situ* gel loaded with paeonol-solid lipid nanoparticles for intranasal drug delivery. *Int. J. Nanomed.,* **2020**, *15*, 3137-3160. http://dx.doi.org/10.2147/IJN.S247935 PMID: 32440115

[93] Scherließ, R. Nasal formulations for drug administration and characterization of nasal preparations in drug delivery. *Ther. Deliv.,* **2020**, *11*(3), 183-191. http://dx.doi.org/10.4155/tde-2019-0086 PMID: 32046624

[94] Shah, B.M.; Misra, M.; Shishoo, C.J.; Padh, H. Nose to brain microemulsion-based drug delivery system of rivastigmine: Formulation and *ex-vivo* characterization. *Drug Deliv.,* **2015**, *22*(7), 918-930. http://dx.doi.org/10.3109/10717544.2013.878857 PMID: 24467601

[95] Soares, S.; Sousa, J.; Pais, A.; Vitorino, C. Nanomedicine: Principles, properties, and regulatory issues. *Front Chem.,* **2018**, *6*, 360. http://dx.doi.org/10.3389/fchem.2018.00360 PMID: 30177965

[96] Bahadur, S.; Pardhi, D.M.; Rautio, J.; Rosenholm, J.M.; Pathak, K. Intranasal nanoemulsions for direct nose-to-brain delivery of actives for cns disorders. *Pharmaceutics,* **2020**, *12*(12), 1230. http://dx.doi.org/10.3390/pharmaceutics12121230 PMID: 33352959

[97] Choi, J-Y.; Park, C.H.; Lee, J. Effect of polymer molecular weight on nanocomminution of poorly soluble drug. *Drug Deliv*., **2008**, *15*(5), 347-353. http://dx.doi.org/101080/10717540802039113

[98] Agrahari, V; Burnouf, P-A; Burnouf, T; Agrahari, V. Nanoformulation properties, characterization, and behavior in complex biological matrices: Challenges and opportunities for brain-targeted drug delivery applications and enhanced translational potential. *Adv. Drug Deliv. Rev.*, **2019**, *148*, 146-180.

[99] Dey, S.; Mahanti, B.; Mazumder, B.; Malgope, A.; Sandeepan, A. Nasal drug delivery: An approach of drug delivery through nasal route. *Der Chem Sin.,* **2011**, *2*(3), 94-106.

[100] Donovan, M.D.; Huang, Y. Large molecule and particulate uptake in the nasal cavity: The effect of size on nasal absorption. *Adv. Drug Deliv. Rev.,* **1998**, *29*(1-2), 147-155. http://dx.doi.org/10.1016/S0169-409X(97)00066-5 PMID: 10837585

[101] Farzal, Z.; Basu, S.; Burke, A.; Fasanmade, O.O.; Lopez, E.M.; Bennett, W.D.; Ebert, C.S., Jr; Zanation, A.M.; Senior, B.A.; Kimbell, J.S. Comparative study of simulated nebulized and spray par-

ticle deposition in chronic rhinosinusitis patients. *Int. Forum Allergy Rhinol.,* **2019**, *9*(7), 746-758.
http://dx.doi.org/10.1002/alr.22324 PMID: 30821929

[102] Kaur, P.; Garg, T.; Rath, G.; Goyal, A.K. *In situ* nasal gel drug delivery: A novel approach for brain targeting through the mucosal membrane. *Artif. Cells Nanomed. Biotechnol.,* **2016**, *44*(4), 1167-1176.
PMID: 25749276

[103] Chand, P.; Pratibha; Gnanarajan, G.; Kothiyal, P. *In situ* gel: A review. *Indian J. Pharm. Biol. Res.,* **2016**, *4*(2), 11-19. [IJPBR].
http://dx.doi.org/10.30750/ijpbr.4.2.2

[104] Prasad, K.M.; Ravindranath, B.S.; Sheetal, B.G. Nasal insitu gel: A novel approach for nasal drug delivery system. *World J. Pharm. Res.,* **2015**, *4*(2), 686-708.

[105] Mujawar, N.; Ghatage, S.; Navale, S.; Sankpal, B.; Patil, S.; Patil, S. Nasal drug delivery: Problem solution and its application. *J. Curr. Pharma Res.,* **2014**, *4*(3), 1231-1245.
http://dx.doi.org/10.33786/JCPR.2014.v04i03.008

[106] Pendolino, A.L.; Lund, V.J.; Nardello, E.; Ottaviano, G. The nasal cycle: A comprehensive review. *Rhinol. Online,* **2018**, *1*(1), 67-76.
http://dx.doi.org/10.4193/RHINOL/18.021

[107] Schipper, N.G.M.; Verhoef, J.C.; Merkus, F.W.H.M. The nasal mucociliary clearance: Relevance to nasal drug delivery. *Pharm. Res.,* **1991**, *8*(7), 807-814.
http://dx.doi.org/10.1023/A:1015830907632 PMID: 1924131

[108] Trenkel, M; Scherließ, R. Nasal powder formulations: *In-vitro* characterisation of the impact of powders on nasal residence time and sensory effects. *Pharm,* **2021**, *13*(3), 385.

[109] Sharma, S.; Lohan, S.; Murthy, R.S.R. Formulation and characterization of intranasal mucoadhesive nanoparticulates and thermoreversible gel of levodopa for brain delivery. *Drug Dev. Ind. Pharm.,* **2014**, *40*(7), 869-878.
http://dx.doi.org/10.3109/03639045.2013.789051 PMID: 23600649

[110] Inoue, D.; Furubayashi, T.; Tanaka, A.; Sakane, T.; Sugano, K. Quantitative estimation of drug permeation through nasal mucosa using *in vitro* membrane permeability across Calu-3 cell layers for predicting *in vivo* bioavailability after intranasal administration to rats. *Eur. J. Pharm. Biopharm.,* **2020**, *149*, 145-153.
http://dx.doi.org/10.1016/j.ejpb.2020.02.004 PMID: 32057906

[111] Kürti, L.; Veszelka, S.; Bocsik, A.; Ózsvári, B.; Puskás, L.G.; Kittel, Á.; Szabó-Révész, P.; Deli, M.A. Retinoic acid and hydrocortisone strengthen the barrier function of human RPMI 2650 cells, a model for nasal epithelial permeability. *Cytotechnology,* **2013**, *65*(3), 395-406.
http://dx.doi.org/10.1007/s10616-012-9493-7 PMID: 22940916

[112] Zada, M.H.; Kubek, M.; Khan, W.; Kumar, A.; Domb, A. Dispersible hydrolytically sensitive nanoparticles for nasal delivery of thyrotropin releasing hormone (TRH). *J. Control. Release,* **2019**, *295*, 278-289.
http://dx.doi.org/10.1016/j.jconrel.2018.12.050 PMID: 30610951

[113] Mistry, A.; Stolnik, S.; Illum, L. Nose-to-brain delivery: Investigation of the transport of nanoparticles with different surface characteristics and sizes in excised porcine olfactory epithelium. *Mol. Pharm.,* **2015**, *12*(8), 2755-2766.
http://dx.doi.org/10.1021/acs.molpharmaceut.5b00088 PMID: 25997083

[114] Ladel, S; Schlossbauer, P; Flamm, J; Luksch, H; Mizaikoff, B; Schindowski, K. Improved *in vitro* model for intranasal mucosal drug delivery: Primary olfactory and respiratory epithelial cells compared with the permanent nasal cell line RPMI 2650. *Pharm,* **2019**, *11*(8), 367.

[115] Na, K.; Lee, M.; Shin, H.W.; Chung, S. *In vitro* nasal mucosa gland-like structure formation on a chip. *Lab Chip,* **2017**, *17*(9), 1578-1584.
http://dx.doi.org/10.1039/C6LC01564F PMID: 28379223

[116] Nižić Nodilo, L.; Ugrina, I.; Špoljarić, D.; Amidžić Klarić, D.; Jakobušić Brala, C.; Perkušić, M.; Pepić, I.; Lovrić, J.; Saršon, V.; Safundžić Kučuk, M.; Zadravec, D.; Kalogjera, L.; Hafner, A. A dry powder platform for nose-to-brain delivery of dexamethasone: Formulation development and nasal deposition studies. *Pharmaceutics,* **2021**, *13*(6), 795.
http://dx.doi.org/10.3390/pharmaceutics13060795 PMID: 34073500

[117] Trenfield, SJ; Awad, A; Madla, CM; Hatton, GB; Firth, J; Goyanes, A Shaping the future: Recent advances of 3D printing in drug delivery and healthcare. **2019**, *16*(10), 1081-1094.

[118] Lungare, S.; Bowen, J.; Badhan, R. Development and evaluation of a novel intranasal spray for the delivery of amantadine. *J. Pharm. Sci.,* **2016**, *105*(3), 1209-1220.
http://dx.doi.org/10.1016/j.xphs.2015.12.016 PMID: 26886345

[119] Ahmad, J.; Haider, N.; Khan, M.A.; Md, S.; Alhakamy, N.A.; Ghoneim, M.M.; Alshehri, S.; Sarim Imam, S.; Ahmad, M.Z.; Mishra, A. Novel therapeutic interventions for combating Parkinson's disease and prospects of Nose-to-Brain drug delivery. *Biochem. Pharmacol.,* **2022**, *195*, 114849.
http://dx.doi.org/10.1016/j.bcp.2021.114849 PMID: 34808125

[120] Khan, S.; Patil, K.; Bobade, N.; Yeole, P.; Gaikwad, R. Formulation of intranasal mucoadhesive temperature-mediated *in situ* gel containing ropinirole and evaluation of brain targeting efficiency in rats. *J. Drug Target.,* **2010**, *18*(3), 223-234.
http://dx.doi.org/10.3109/10611860903386938 PMID: 20030503

[121] Rao, M.; Agrawal, D.K.; Shirsath, C. Thermoreversible mucoadhesive *in situ* nasal gel for treatment of Parkinson's disease. *Drug Dev. Ind. Pharm.,* **2017**, *43*(1), 142-150.
http://dx.doi.org/10.1080/03639045.2016.1225754 PMID: 27533244

[122] Ravi, P.R.; Aditya, N.; Patil, S.; Cherian, L. Nasal *in-situ* gels for delivery of rasagiline mesylate: Improvement in bioavailability and brain localization. *Drug Deliv.,* **2015**, *22*(7), 903-910.
http://dx.doi.org/10.3109/10717544.2013.860501 PMID: 24286183

[123] Djupesland, P.G.; Messina, J.C.; Mahmoud, R.A. Role of nasal casts for *in vitro* evaluation of nasal drug delivery and quantitative evaluation of various nasal casts. In: *Therapeutic Delivery*; Newlands Press Ltd London: UK, **2020**; pp. 485-495.
http://dx.doi.org/10.4155/tde-2020-0054

[124] Samoliński, B.K.; Grzanka, A.; Gotlib, T. Changes in nasal cavity dimensions in children and adults by gender and age. *Laryngoscope,* **2007**, *117*(8), 1429-1433.
http://dx.doi.org/10.1097/MLG.0b013e318064e837 PMID: 17607151

[125] Warnken, Z.N.; Smyth, H.D.C.; Davis, D.A.; Weitman, S.; Kuhn, J.G.; Williams, R.O., III; Williams, I. Personalized medicine in nasal delivery: The use of patient-specific administration parameters to improve nasal drug targeting using 3D-printed nasal replica casts. *Mol. Pharm.,* **2018**, *15*(4), 1392-1402.
http://dx.doi.org/10.1021/acs.molpharmaceut.7b00702 PMID: 29485888

[126] Kundoor, V; Dalby, RN Assessment of nasal spray deposition pattern in a silicone human nose model using a color-based method. *Pharm Res,* **2009**, *27*(1), 30-36.

[127] Kiaee, M.; Wachtel, H.; Noga, M.L.; Martin, A.R.; Finlay, W.H. An idealized geometry that mimics average nasal spray deposition in adults: A computational study. *Comput. Biol. Med.,* **2019**, *107*, 206-217.
http://dx.doi.org/10.1016/j.compbiomed.2019.02.013 PMID: 30851506

[128] Vachhani, S.; Kleinstreuer, C. Comparison of micron- and nanoparticle transport in the human nasal cavity with a focus on the olfactory region. *Comput. Biol. Med.,* **2021**, *128*, 104103.
http://dx.doi.org/10.1016/j.compbiomed.2020.104103 PMID: 33220592

[129] Shah, V.; Sharma, M.; Pandya, R.; Parikh, R.K.; Bharatiya, B.; Shukla, A.; Tsai, H.C. Quality by design approach for an *in situ* gelling microemulsion of Lorazepam *via* intranasal route. *Mater. Sci. Eng. C,* **2017**, *75*, 1231-1241.
http://dx.doi.org/10.1016/j.msec.2017.03.002 PMID: 28415411

[130] Ladel, S; Flamm, J; Zadeh, AS; Filzwieser, D; Walter, J-C; Schlossbauer, P Allogenic Fc domain-facilitated uptake of IgG in nasal lamina propria: Friend or foe for intranasal CNS delivery? *Pharm,* **2018**, *10*(3), 107.

[131] Khafaji, AS; Al, ; Donovan, MD Endocytic uptake of solid lipid nanoparticles by the nasal mucosa. *Pharm,* **2021**, *13*(5), 761.
http://dx.doi.org/10.3390/pharmaceutics13050761

[132] Kanazawa, T; Fukuda, M; Suzuki, N; Suzuki, T. Novel methods for intranasal administration under inhalation anesthesia to evaluate

nose-to-brain drug delivery. *J. Vis. Exp.,* **2018**, *2018*(141), p.e58485.

[133] Tolosa, E.; Martí, M.J.; Valldeoriola, F.; Molinuevo, J.L. History of levodopa and dopamine agonists in Parkinson's disease treatment. *Neurology,* **1998**, *50*(Suppl. 6), S2-S10. http://dx.doi.org/10.1212/WNL.50.6_Suppl_6.S2 PMID: 9633679

[134] Kao, H.D.; Traboulsi, A.; Itoh, S.; Dittert, L.; Hussain, A. Enhancement of the systemic and CNS specific delivery of L-dopa by the nasal administration of its water soluble prodrugs. *Pharm. Res.,* **2000**, *17*(8), 978-984. http://dx.doi.org/10.1023/A:1007583422634 PMID: 11028945

[135] Samaridou, E.; Walgrave, H.; Salta, E.; Álvarez, D.M.; Castro-López, V.; Loza, M.; Alonso, M.J. Nose-to-brain delivery of enveloped RNA - cell permeating peptide nanocomplexes for the treatment of neurodegenerative diseases. *Biomaterials,* **2020**, *230*, 119657. http://dx.doi.org/10.1016/j.biomaterials.2019.119657 PMID: 31837821

[136] Agrawal, M.; Saraf, S.; Saraf, S.; Dubey, S.K.; Puri, A.; Gupta, U.; Kesharwani, P.; Ravichandiran, V.; Kumar, P.; Naidu, V.G.M.; Murty, U.S.; Ajazuddin, ; Alexander, A. Stimuli-responsive *in situ* gelling system for nose-to-brain drug delivery. *J. Control. Release,* **2020**, *327*, 235-265. http://dx.doi.org/10.1016/j.jconrel.2020.07.044 PMID: 32739524

[137] Hoare, T.R.; Kohane, D.S. Hydrogels in drug delivery: Progress and challenges. *Polymer (Guildf.),* **2008**, *49*(8), 1993-2007. http://dx.doi.org/10.1016/j.polymer.2008.01.027

[138] Sosnik, A.; Seremeta, K. Polymeric hydrogels as technology platform for drug delivery applications. *Gels,* **2017**, *3*(3), 25. http://dx.doi.org/10.3390/gels3030025 PMID: 30920522

[139] Aderibigbe, B.A. *In situ*-based gels for nose to brain delivery for the treatment of neurological diseases. *Pharmaceutics,* **2018**, *10*(2), 40. http://dx.doi.org/10.3390/pharmaceutics10020040

[140] Overcoming Parkinson's disease: Direct nose-to-brain delivery of amantadine. *Open Research Online,* Available from: http://oro.open.ac.uk/43400/ [Accessed on: 2022 Jun, 24].

[141] Hoban, D.B.; Newland, B.; Moloney, T.C.; Howard, L.; Pandit, A.; Dowd, E. The reduction in immunogenicity of neurotrophin overexpressing stem cells after intra-striatal transplantation by encapsulation in an *in situ* gelling collagen hydrogel. *Biomaterials,* **2013**, *34*(37), 9420-9429. http://dx.doi.org/10.1016/j.biomaterials.2013.08.073 PMID: 24054846

[142] Khafagy, E.S.; Kamei, N.; Fujiwara, Y.; Okumura, H.; Yuasa, T.; Kato, M.; Arime, K.; Nonomura, A.; Ogino, H.; Hirano, S.; Sugano, S.; Takeda-Morishita, M. Systemic and brain delivery of leptin *via* intranasal coadministration with cell-penetrating peptides and its therapeutic potential for obesity. *J. Control. Release,* **2020**, *319*, 397-406. http://dx.doi.org/10.1016/j.jconrel.2020.01.016 PMID: 31926192

[143] Aly, A.E-E.; Waszczak, B.L. Intranasal gene delivery for treating Parkinsons disease: Overcoming the blood-brain barrier. *Exp. Opin. Drug Deliv. Informa Healthcare,* **2015**, *12*, 1923-1941.

[144] Dhuria, S.V.; Hanson, L.R.; Frey, W.H., II Intranasal delivery to the central nervous system: Mechanisms and experimental considerations. *J. Pharm. Sci.,* **2010**, *99*(4), 1654-1673. http://dx.doi.org/10.1002/jps.21924 PMID: 19877171

[145] Malerba, F.; Paoletti, F.; Capsoni, S.; Cattaneo, A. Intranasal delivery of therapeutic proteins for neurological diseases. *Expert Opin. Drug Deliv.,* **2011**, *8*(10), 1277-1296. http://dx.doi.org/10.1517/17425247.2011.588204 PMID: 21619468

[146] Lochhead, J.J.; Thorne, R.G. Intranasal delivery of biologics to the central nervous system. *Adv. Drug Deliv. Rev.,* **2012**, *64*(7), 614-628. http://dx.doi.org/10.1016/j.addr.2011.11.002 PMID: 22119441

[147] Lu, C.T.; Jin, R.R.; Jiang, Y.N.; Lin, Q.; Yu, W.Z.; Mao, K.L.; Tian, F.R.; Zhao, Y.P.; Zhao, Y.Z. Gelatin nanoparticle-mediated intranasal delivery of substance P protects against 6-hydroxydopamine-induced apoptosis: An *in vitro* and *in vivo* study. *Drug Des. Devel. Ther.,* **2015**, *9*, 1955-1962. PMID: 25897205

[148] Samaridou, E.; Alonso, M.J.; María, J.A.; Epithelium, O. Nose-to-brain peptide delivery-the potential of nanotechnology. *Bioorg. Med. Chem*., **2018**, *26*(10), 2888-2905.

[149] Decressac, M.; Pain, S.; Chabeauti, P.Y.; Frangeul, L.; Thiriet, N.; Herzog, H.; Vergote, J.; Chalon, S.; Jaber, M.; Gaillard, A. Neuroprotection by neuropeptide Y in cell and animal models of Parkinson's disease. *Neurobiol. Aging,* **2012**, *33*(9), 2125-2137. http://dx.doi.org/10.1016/j.neurobiolaging.2011.06.018 PMID: 21816512

[150] Duarte-Neves, J; Cavadas, C; Pereira de Almeida, L. Neuropeptide Y (NPY) intranasal delivery alleviates Machado-Joseph disease. *Sci Reports,* **2021**, *11*(1), 1-9.

[151] Intranasal administration of neuropeptide Y in healthy male volunteers - full text view. *ClinicalTrials.gov*.

[152] Mathé, A.A.; Michaneck, M.; Berg, E.; Charney, D.S.; Murrough, J.W. A randomized controlled trial of intranasal neuropeptide Y in patients with major depressive disorder. *Int. J. Neuropsychopharmacol.,* **2020**, *23*(12), 783-790. http://dx.doi.org/10.1093/ijnp/pyaa054 PMID: 33009815

[153] Noè, F.; Pool, A.H.; Nissinen, J.; Gobbi, M.; Bland, R.; Rizzi, M.; Balducci, C.; Ferraguti, F.; Sperk, G.; During, M.J.; Pitkänen, A.; Vezzani, A. Neuropeptide Y gene therapy decreases chronic spontaneous seizures in a rat model of temporal lobe epilepsy. *Brain,* **2008**, *131*(6), 1506-1515. http://dx.doi.org/10.1093/brain/awn079 PMID: 18477594

[154] Fiebich, B.L.; Schleicher, S.; Butcher, R.D.; Craig, A.; Lieb, K. The neuropeptide substance P activates p38 mitogen-activated protein kinase resulting in IL-6 expression independently from NF-κ B. *J. Immunol.,* **2000**, *165*(10), 5606-5611. http://dx.doi.org/10.4049/jimmunol.165.10.5606 PMID: 11067916

[155] Zhao, Y.Z.; Jin, R.R.; Yang, W.; Xiang, Q.; Yu, W.Z.; Lin, Q.; Tian, F.R.; Mao, K.L.; Lv, C.Z.; Wáng, Y.X.J.; Lu, C.T. Using gelatin nanoparticle mediated intranasal delivery of neuropeptide substance P to enhance neuro-recovery in hemiparkinsonian rats. *PLoS One,* **2016**, *11*(2), e0148848. http://dx.doi.org/10.1371/journal.pone.0148848 PMID: 26894626

[156] Rizk, S.S.; Misiura, A.; Paduch, M.; Kossiakoff, A.A. Substance P derivatives as versatile tools for specific delivery of various types of biomolecular cargo. *Bioconjug. Chem.,* **2012**, *23*(1), 42-46. http://dx.doi.org/10.1021/bc200496e PMID: 22175275

[157] Ding, G.; Wang, T.; Han, Z.; Tian, L.; Cheng, Q.; Luo, L.; Zhao, B.; Wang, C.; Feng, S.; Wang, L.; Meng, Z.; Meng, Q. Substance P containing peptide gene delivery vectors for specifically transfecting glioma cells mediated by a neurokinin-1 receptor. *J. Mater. Chem. B Mater. Biol. Med.,* **2021**, *9*(32), 6347-6356. http://dx.doi.org/10.1039/D1TB00577D PMID: 34251002

[158] Bender, T.S.; Migliore, M.M.; Campbell, R.B.; John Gatley, S.; Waszczak, B.L. Intranasal administration of glial-derived neurotrophic factor (GDNF) rapidly and significantly increases whole-brain GDNF level in rats. *Neuroscience,* **2015**, *303*, 569-576. http://dx.doi.org/10.1016/j.neuroscience.2015.07.016 PMID: 26166725

[159] Kordower, J.H.; Bjorklund, A. Trophic factor gene therapy for Parkinson's disease. *Mov. Disord.,* **2013**, *28*(1), 96-109. http://dx.doi.org/10.1002/mds.25344 PMID: 23390096

[160] Murlidharan, G.; Samulski, R.J.; Asokan, A. Biology of adeno-associated viral vectors in the central nervous system. *Front. Mol. Neurosci.,* **2014**, *7*, 76. http://dx.doi.org/10.3389/fnmol.2014.00076 PMID: 25285067

[161] Tenenbaum, L.; Chtarto, A.; Lehtonen, E.; Velu, T.; Brotchi, J.; Levivier, M.Recombinant AAV-mediated gene delivery to the central nervous system. *J Gene Med,* **2004**, *6*(Suppl 1), S212-S222.

[162] McCully, J.D.; Cowan, D.B.; Emani, S.M.; del Nido, P.J. Mitochondrial transplantation: From animal models to clinical use in humans. *Mitochondrion,* **2017**, *34*, 127-134. http://dx.doi.org/10.1016/j.mito.2017.03.004 PMID: 28342934

[163] Chang, J-C; Chao, Y-C; Chang, H-S; Wu, Y-L; Chang, H-J; Lin, Y-S Intranasal delivery of mitochondria for treatment of Parkinson's Disease model rats lesioned with 6-hydroxydopamine. *Sci Reports,* **2021**, *11*(1), 1-14.

[164] Alexander, J.F.; Seua, A.V.; Arroyo, L.D.; Ray, P.R.; Wangzhou, A.; Heiβ-Lückemann, L.; Schedlowski, M.; Price, T.J.; Kavelaars,

A.; Heijnen, C.J. Nasal administration of mitochondria reverses chemotherapy-induced cognitive deficits. *Theranostics,* **2021**, *11*(7), 3109-3130. http://dx.doi.org/10.7150/thno.53474 PMID: 33537077

[165] Chen, H.; Yang, G.Z.X.; Getachew, H.; Acosta, C.; Sierra Sánchez, C.; Konofagou, E.E. Focused ultrasound-enhanced intranasal brain delivery of brain-derived neurotrophic factor. *Sci. Rep.,* **2016**, *6*(1), 28599. http://dx.doi.org/10.1038/srep28599 PMID: 27345430

[166] Ji, R.; Smith, M.; Niimi, Y.; Karakatsani, M.E.; Murillo, M.F.; Jackson-Lewis, V.; Przedborski, S.; Konofagou, E.E. Focused ultrasound enhanced intranasal delivery of brain derived neurotrophic factor produces neurorestorative effects in a Parkinson's disease mouse model. *Sci. Rep.,* **2019**, *9*(1), 19402. http://dx.doi.org/10.1038/s41598-019-55294-5 PMID: 31852909