Recent advances in delivery of peptide and protein therapeutics to the brain

Sanchit Arora\textsuperscript{a\textdagger}, Tania Bajaj\textsuperscript{b\textdagger}, Jayant Kumar\textsuperscript{c}, Manoj Goyal\textsuperscript{c}, Arti Singh\textsuperscript{d}, Charan Singh\textsuperscript{c}

\textsuperscript{a}Department of Pharmaceutics, Delhi Pharmaceutical Sciences & Research University (DPSRU), Mehrauli-Badarpur Road, New Delhi-110017, India
\textsuperscript{b}Department of Pharmaceutics, ISF College of Pharmacy, Moga-142001, Punjab, India
Affiliated to I.K. Gujral Punjab Technical University, formerly Punjab Technical University, Kapurthala Jalandhar-144603, Punjab, India
\textsuperscript{c}Department of Pharmaceutical Sciences, School of Sciences, Hemvati Nandan Bahuguna Garhwal University (A Central University), Srinagar Garhwal-246174, Uttarakhand, India
\textsuperscript{d}Department of Pharmacy, School of Health Sciences, Central University of South Bihar, Gaya-824236, Bihar, India
\textsuperscript{e}Department of Pharmacology, ISF College of Pharmacy, Moga-142001, Punjab, India
Affiliated to I.K. Gujral Punjab Technical University, formerly Punjab Technical University, Kapurthala Jalandhar-144603, Punjab, India

\textdagger}Equally contributing author

*Corresponding author
Dr. Charan Singh (PhD)
Assistant Professor
Email: c.singhniper009@gmail.com
Abstract

The classes of neuropharmaceuticals known as proteins and peptides serve as diagnostic tools and are involved in specific communication in the peripheral and central nervous systems (CNS). However, due to tight junctions resembling epithelial cells found in the blood-brain barrier (BBB) in vivo, they are typically excluded from transport from the blood to the brain. The drugs having molecular weight of less than 400 Dalton are able to cross the BBB via lipid-mediated free diffusion. However, large molecule therapeutics are devoid of these characteristics. As an alternative, these substances may be carried via chimeric peptide drug delivery systems, and assist in transcytosis through BBB with the aid of linker strategies. With their recent developments, several forms of nanoparticles, including PEG-PCL, nanogels, liposomes, NLCs, PLGA-NPs, chitosan, and SLNs, have also been considered for their therapeutic applications. Moreover, the necessity for physiological optimization of current drug delivery methods and their carriers to deliver therapeutic doses of medication into the brain for the treatment of various neurological illnesses has also been emphasized. Therapeutic use of proteins and peptides has no neuroprotective impact in the absence of all these methods. Each tactic, however, has unique drawbacks and considerations. In this review, we discuss different drug delivery methods for therapeutic distribution of pharmaceuticals, primarily neuroproteins and neuropeptides, through endothelial capillaries via blood-brain barrier. Finally, we have also discussed challenges and future perspective of protein and peptide therapeutics delivery to the brain.

Keywords

Proteins, Peptides, Therapeutics, Vectors, Brain delivery, Central Nervous System

Significance Statement

Very few reports on the delivery of therapeutic protein and peptide nanoformulations are available in the literature. Herein, we attempted to discuss these nanoformulations of protein and peptide therapeutics used to treat brain diseases.
List of non-standard Abbreviations

DGL-PLGA-angiopep/hGDNF nanoparticles: Dendrigraft poly-L-lysine conjugated PLGA angiopep loaded human glial cell line-derived neurotrophic factor nanoparticles, DOX@R8PLP: Doxorubicin loaded liposomal formulation anchored with octaarginine, Lf@PEG-PCL NPs: Lactoferrin loaded poly (ethylene glycol)-poly(ε-caprolactone) copolymers nanoparticles, RVG29/TPP-MA-SLN-GS: Genistein loaded with macrophage membrane-coated SLN and modified it with the mitochondria-targeting molecule TPP and rabies virus glycoprotein, Vit D-BP-PLGA NPs: Vitamin D-binding protein loaded PLGA nanoparticles
Running Title page

Running Title: Protein and peptide therapeutics brain delivery
1. Introduction

Although the sector of peptide and protein therapeutics has grown significantly in recent decades, their delivery to the brain has limited their clinical applicability (Wang et al., 2022). Due to the lower penetration of the blood-brain barrier (BBB) by large molecule drugs such as peptide and protein drugs in the brain, the discovery of innovative drugs to treat neurological disorders has been slow (Pardridge, 2019). Currently, the physical methods are used to transport the heavy molecules to bypass various cell-based challenges such as the blood-brain barrier, drug transport pumps and tight endothelial junctions. However, direct injection can overcome these challenges to deliver the drug to the central nervous system (CNS) (Upadhyay, 2014). Despite improvements in technology and knowledge, the physical deliveries of protein molecules for brain diseases continue to suffer from serious deficiencies. The physical pathways are risky, destructive, have no cerebral distraction, and do not treat the symptoms. Therefore, it is essential to explore new elegant, beneficial and gentle delivery strategies (Bellettato and Scarpa, 2018).

Therefore, there is an urgent need for novel delivery approaches that can effectively deliver the protein and peptide molecules without disturbing the tight junction or brain barriers (Li et al., 2021). It's worth noting that the brain refuses to allow large molecules inside. However, only a few molecules such as insulin, transferrin and ceruloplasmin are required for the brain, even if they have higher molecular weight and can easily cross the endothelial barrier such as the BBB. Their transmission could be possible due to the presence of receptor-mediated transport (RMT) in the blood-brain barrier, such as the insulin receptor (IR), the transferrin receptor (TfR) and the low-density lipoprotein receptor (LDLR) (Pulgar, 2018). Most biological drugs (recombinant proteins, therapeutic antibodies, nucleic acid) are large molecules that cannot enter the CNS (Vargason and Anselmo, 2021). Nevertheless, the idea is to deliver protein and peptide drugs into the CNS by anchoring the targeting moiety on the surface of the nanocarriers. It is reported that some peptides, for example insulin or transfer peptides, can help deliver the drugs to the brain via IR and TfR, respectively. Another mechanism is adsorptive-mediated transcytosis (AMT), which allows therapeutic uptake inside the brain (Jones and Shusta, 2007). Various cellular mechanisms of high molecular weight therapeutics absorption via BBB are shown in the Fig. 1.
There are several mechanisms via AMT that allow the binding and adsorption of positively charged molecules to the luminal part of the brain, followed by exocytosis on the abluminal surface (Herv et al., 2008).

Recently, there have been numerous attempts to target the peptide and protein drugs to the brain using nanotechnology-enabled drug delivery systems. Some of these include lipids and polymer carriers such as liposomes, solid lipid nanoparticles and nanostructured lipid nanoparticles. In this regard, polymeric NPs have clear advantages over other carrier systems (Su and P, 2020). Polymeric NPs are a suitable drug carrier for parenteral injection due to their smaller size vis-à-vis microparticles. Furthermore, NPs have been shown to move through the BBB and tight junctions more effectively than microparticles (Mohammed et al., 2017). Nano drug delivery technology offers unique advantages for drug delivery. Numerous physicochemical properties such as particle size, shape, hydrophilicity and surface charge play an important role in improving the biopharmaceutical attributes of the drugs (Sultana et al., 2022). Furthermore, novel drug delivery systems have a unique drug release pattern that increases the availability of the drug at the local site of action while decreasing it at the non-target site, thereby reducing the occurrence of potential side effects (Tewabe and Abate, 2021).

This review mainly focuses on the pathways for the transport of the protein and peptide therapeutics for the brain disorders such as Alzheimer’s disease, Parkinson’s disease, epilepsy, neuropathic pain and autism spectrum disorders. Moreover, numerous linker strategies such as disulfide and avidin (biotin, fusion) have also been reviewed. Additionally, it touches upon the role of various absorptive and receptor mediated vectors in the delivery of drugs. Here, we have also discussed in detail the advanced formulations of protein and peptide therapeutics.

2. Pathways for the transport of proteins and peptides across the blood brain barrier

There are multiple transport pathways through which peptides and protein therapeutics could be delivering across the BBB; these include diffusion, carrier mediated transport (CMT), receptor mediated endocytosis (RME), and absorptive-mediated transcytosis. The increase in lipophilicity is necessary for diffusion, while RMT and CMT absorption requires a transport-specific ligand (Tewabe and Abate, 2021). In this review we have focussed mainly on two strategies first is absorptive-mediated vectors and receptor-mediated vectors. Adsorptive-mediated transcytosis (AMT) allows medications to cross the BBB and enable entry to the brain. The BBB is perfectly suited for the AMT process because it offers the possibility of cationic molecule binding and
absorption to the luminal surface of endothelial cells, followed by exocytosis at the abluminal surface (Song and Lu, 2021).

Drug delivery of the absorption vectors to the brain might be possible using cationized albumin and cationized immunoglobulin. However, in receptor-mediated endocytosis, ligands attach to specific receptors present on the BBB. There are several endogenous transport mechanisms such as L-type amino acid transporter1 receptor which transports amino acids. On the other hand, glucose transporter1 receptor transports glucose, the brain’s primary energy source (Xiao and Gan, 2013). Apart from this, insulin-like growth factors (IGF-I, IGF-II) and leptin are carried by endogenous receptors found in endothelium cells that makes up the BBB. In addition, the other molecules such as thiamine, biotin, folic acid, vitamin B12, Tf and neuropeptides are transported by their respective specialized receptors (Lewitt and Boyd, 2019).

For example, glucose is an important source of energy for the brain and is transported by the glucose transporter, while BBB also expresses several amino acid transporter-1 receptors that allow amino acids to enter the brain. This is because amino acids are also required for the overall development and production of neurotransmitters (Navale and Paranjape, 2016). However, the larger molecules such as insulin, leptin, folate transferrin and neuropeptides can easily cross the endothelial barrier through their respective specialized receptors (Rhea and Rask-Madsen, 2018).

**Figure 2** depicts the many receptors that primarily transfer proteins and peptides into the brain via receptor-mediated transcytosis.

### 2.2 Receptor mediated vectors

RMT is an appealing approach for peptide and protein administration since this vesicle-based strategy enables the migration of a diverse spectrum of endogenous macromolecules from blood to the brain with high specificity, selectivity, and affinity (Wang et al., 2015). RMT can be referred to as clathrin-dependent endocytosis because the membrane-associated protein clathrin helps to create membrane vesicles that are internalized inside the cell (Hansen and Nichols, 2009). Clathrin-coated vesicle development occurs in five steps, each of which corresponds to ultrastructural and cell biology observations: initiation, cargo selection, coat construction, scission, and uncoating (Hansen and Nichols, 2009). Clathrin cannot directly connect to the membrane or cargo receptors. It requires adaptor proteins and complexes (like adaptor protein 2 (AP2) and supplementary proteins (like AP180 and epsin) (Royle, 2006). Subsequent binding of
antigen to the receptor, the protein complex diffuses laterally through the plasma membrane until it comes across a specific membrane region known as a coated pit. In addition to other proteins like clathrin, adaptor protein, and dynamin, the receptor-ligand complexes and other proteins also build up in these patches (Mettlen et al., 2018). Coated pits are not significant membrane characteristics because they take up roughly 20% of the plasma membrane's surface area. As a result of the accumulation of these proteins, the membrane's nearby portion begins to curl and finally pinches off to produce an internalized coated vesicle (Moore et al., 1987). The uncoated vesicle is thus free to merge with an early endosome when clathrin and dynamin recycle back to the plasma membrane. The lysosome is where the early endosomes go for digestion once they develop into late endosomes (Grant and Donaldson, 2009). There have been two key strategies that can be used for the manufacturing of RMT-targeting biologics. In the first strategy, a fusion protein or chemical linkage, such as the streptavidin/biotin linkage, can be used to bind the RMT targeting moiety and biologic together (Jones and Shusta, 2007; Lajoie and Shusta, 2015). The second method is creating liposomes or polymeric nanoparticles that are loaded with the desired biologic and coated with RMT targeting ligands (Pinheiro and Coutinho, 2021). The following part mostly discusses protein and peptide transport mediated by insulin and transferrin receptors.

2.2.1 Insulin and insulin-like growth factors

Over a period of 30 years, insulin, IGF 1, IGF 2 and their receptors have gained the tremendous attraction in terms of growth and functioning of the CNS (Biadgo et al., 2020). There is insulin receptor throughout the brain; however, the density of expression varies from region to region in the brain. Increased expression is present in the pyriform cortex, thalamus, amygdaloid complex, olfactory regions and hippocampus. Importantly, IR is most strongly expressed in the dorsomedial, supraoptic, and arcuate nuclei of the hypothalamus (Werner and LeRoith, 2014). Insulin is a peptide and has a higher molecular weight, nonetheless due to presence of these receptors, insulin can enter in the brain (Banks et al., 2012). Insulin, IGF-1, IGF-2 are endogenous ligands of IR (Boucher et al., 2010). All cells must undergo ligand-induced autophosphorylation for IR internalization. After activation, IR autophosphorylates and undergoes endocytosis to enter the cell. In order to initiate further signaling cascades, phosphorylated IR attracts and activates target molecules such as insulin receptor substrates (IRSs), Src homology 2-B (SH2-B), and protein phosphatases (Chen et al., 2019). IR is then regulated in the early endosome (EE), a protein-sorting platform, for subsequent trafficking.
bulk of IRs are recycled back to the plasma membrane after being inactivated and sorted in EE, whereas a minor number are translocated to the late endosome for destruction or the nucleus (Iraburu et al., 2021). This phenomenal brain insulin uptake mechanism gives an idea to deliver therapeutic macromolecules across the BBB using encapsulating the peptide therapeutics in the anti-IR antibodies.

Mucopolysaccharidosis type I (MPSI) is a genetic disorder that affects mainly neurological function and is caused by mutations in the gene encoding the lysosomal enzyme, α-l-iduronidase (IDUA). Enzyme replacement therapy has been suggested for MPSI. However, the brain refuses to accept IDUA. In the present investigation, researchers fused IDUA with a monoclonal antibody (mAb) against the human insulin receptor (HIR). Surprisingly, the data demonstrate that IDUA alone does not travel into the brain, whereas the HIRMAb-IDUA fusion protein easily crosses the BBB (Boado and Pardridge, 2017). Additionally, Hargreaves pain model, systemic injection of IGF1R4-mFc coupled with the non-BBB crossing analgesic peptide galanin (2 and 5 mg/kg) elicited a dose-dependent decrease of heat hyperalgesia. Finally, anti-IGF1R sdAbs demonstrated receptor-mediated brain absorption as well as pharmacologically efficient parenchymal administration of non-permeable neuroactive peptides (Alata et al., 2022). These findings provide an exciting new path for delivering proteins and peptides throughout the brain, as well as hope for neurological patients.

2.2.2 Monoclonal antibodies to the transferrin receptor

Iron is required for several biological processes in the brain, but before it can be used by neuronal cells in the brain, it must cross the BBB (Mills et al., 2010). Transferrin is a key iron-carrying protein in the body, having a molecular weight of 80 kDa and two distinct, high-affinity Fe (III) binding domains (Tandara and Salamunic, 2012). The circulatory free iron first binds to the transferrin, and this iron-loaded transferrin then enables entry to the BBB through binding to the transferrin receptor. The transferrin receptor is a crucial transporter for transferrin (Duck and Connor, 2016). It is necessary for cellular iron uptake and is controlled in response to intracellular iron levels. The receptor-mediated endocytosis is one significant step that aids in the internalization of the transferrin-iron complex (Wang and Pantopoulos, 2011). The peripheral vasculature system has limited expression of TfR while having instant access to moving holo-Tf. However, the endothelial cells of the BBB have a higher expression of TfR (Lajoie and Shusta, 2015). The endocytosis of transferrin is mainly mediated through clathrin-coated pits. An
endosome is formed after the internalization of the transferrin complex. Then the attached molecule is released from the Tf as a result of an endosomal pH decrease. Both the receptor and ligand are subsequently recycled to the plasma membrane (Mayle et al., 2012). Nerve growth factor (NGF) is remarkably reduced in neurodegenerative disorders and is also required for the survival of both peripheral ganglion cells and central cholinergic neurons of the basal forebrain. Friden et al reported that the delivery of NGF through conjugation with antibody to the transferrin receptor. NGF is a huge polypeptide growth factor and cannot pass through the BBB. However, after combining with an anti-transferrin receptor antibody, it is feasible for it to enter the brain. Although they have not investigated the NGF uptake mechanism. They concluded that NGF distribution might improve neuron survival in the AD mouse model. This strategy might be effective for the management of Alzheimer's disease and other neurological disorders that can be treated with proteins that are unable to easily penetrate the BBB (Friden et al., 1993). In an additional study, Pardridge et al. demonstrated the administration of brain-derived neurotrophic factor (BDNF) by ligation using a transferrin receptor antibody (Pardridge et al., 1994). Surprisingly, this technique allows for the delivery of a BDNF-like bigger molecule into the brain.

2.3 Adsorptive-mediated transcytosis (AMT)

A simple electrostatic interaction among the positive charge on the drug formulation surface and the anion on the BBB membrane allows AMT to transport pharmaceuticals inside the brain. The process is unidirectional from blood to brain and is mediated by clathrin-dependent endocytosis (Barar et al., 2016). The process is unidirectional from blood to brain and is mediated by clathrin-dependent endocytosis (Kumagai et al., 1987). A simple cationization of albumin and immunoglobulins can provide an absorptive vector, which is essential for the transport of bigger molecules (Triguero et al., 1991).

2.3.1 Cationized albumin

In the past few decades, protein and peptide therapy has gained lot of attentions in the areas of biomedicine. There are studies on proteins and peptides as therapeutics, which are considered an attractive approach to combat various diseases (Bruno et al., 2013). Unfortunately, they have been associated with few significant technological shortcomings, the most important of which are their high immunogenicity and low safety margin, which limit their use in clinics (Fernandez et al., 2018). Human serum albumin (HSA), a promising macromolecular drug cargo,
has been endowed with several advantages, e.g., being non-immunogenic, biocompatible, non-toxic, stable in plasma and easily metabolized in biological systems (Hong et al., 2020). Furthermore, the metabolites HSA can be easily filtered through the kidney and are hydrophilic. These properties make it an ideal delivery system for the generation of nanodrug delivery of biotherapeutics (Spada et al., 2021). In contrast to native albumin (PI < 4), cationized albumin (PI > 8) penetrates rapidly from the blood into the cerebrospinal fluid (CSF). This, indicates that the BBB, which is the capillary wall of the brain, may have a unique mechanism for cationized albumin absorption. Kumagai et al. reported delivery of the endorphin by coupling it to a transportable peptide, e.g., cationized albumin endorphin is a non-transportable peptide (Kumagai et al., 1987). Kesharwani et al. reported that CBA-conjugated poly (D, L-lactide-co-glycolide) (PLGA) NPs were loaded with methotrexate in brain tumors. This study further suggests that at all doses tested, CBA-bound NPs encapsulating MTX showed more cytotoxicity in C6 glioma cells than pure MTX. The composition of a cancer drug leveraged the BBB to expertly handle large volumes (Kesharwani et al., 2016). Though there have been few literatures available on this topic, based on the existing literature, it could be speculated that instead of the chemical, we can link a protein or peptide with cationized albumin. Similar conjugation strategies may enable the entry of protein or peptide molecules into the brain.

2.3.2 Cationized immunoglobulins

The three major bioengineered products, recombinant proteins, monoclonal antibodies (mAbs), and antisense oligonucleotides, are unlikely to be helpful drugs for the brain until these are safe and effective. The lack of certain transport pathways within the brain blood vessel wall makes it inaccessible to the brain (Achar et al., 2021). However, the cationized IgG could cross the BBB. By interacting with naturally negatively charged plasma membranes, cationized mAb may promote transcellular transport across the BBB. It could be a potential carrier for treatment of neurological diseases as well as for neurodiagnostic imaging. The cationization of IgG might be possible through the permanent attachment of primary NH₂ onto the surface. It is well known that cationizing proteins enhance their overall cellular absorption. Triguero et al. reported that autoradiography of brain slices showed that ¹²⁵I-labeled positively charged IgG molecule had completely crossed the BBB and entered the brain parenchyma compared to the IgG molecule after receiving carotid artery infusions of ¹²⁵I-cationized IgG. Furthermore, the investigator concluded that cationization of IgG molecules greatly aided the transit of these plasma proteins.
across the BBB in-vivo, suggesting a potential strategy for delivering IgG across the BBB (Triguero et al., 1989). Syvänena et al reported that cationization of amyloid-beta (Aβ) protofibril selective F(ab’)2 fragment (F(ab’)2-h158) boost brain concentrations of therapeutic antibodies (Syvänen et al., 2017). This strategy could be used for delivering the peptide linkage with cationized immunoglobulin. However, these are just speculation more detailed investigation is required to validate their therapeutic applicability.

3. Proteins and Peptides therapeutics in various neurological disorders

The development of pharmaceuticals, particularly neuroproteins, and neuropeptides for treating central nervous system (CNS) disorders, faces challenges in penetrating the blood-brain barrier (BBB) to reach their intended targets in the brain. There have been various strategies employed to enhance the delivery of these molecules to the brain through endogenous transport pathways, such as passive diffusion, active transport, and endocytosis. These molecules demonstrate therapeutic properties, such as acting as receptor agonists or antagonists, or actively targeting ligands (Endostatin peptides are recognized for their ability to inhibit angiogenesis. Neurotrophins, such as nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF), are neuroprotective) (Upadhyay et al., 2014). Additionally, proteins and peptides can facilitate physiological functions. For example, cell-penetrating peptides have been developed to mimic viruses by promoting endosomal escape and internalization. Other protein therapies can exploit the inherent cell transport and signaling mechanisms (Stewart et al., 2008). Nano-enabled delivery systems present a promising solution to enhance the uptake and targeted delivery of protein and peptide therapeutics into the brain, among various strategies. Furthermore, these delivery systems offer innovative solutions by utilizing their size and surface properties to facilitate brain penetration (Vashist et al., 2018). Various nanocarriers including nanoparticles, liposomes, and micelles can be used as a means to encapsulate proteins and peptide therapeutics, either alone or in combination with targeting ligands. Furthermore, protecting them from enzymatic degradation and enhances their transport across BBB. Surface modifications, such as PEGylation, can further improve stability and circulation time. Additionally, responsive nanosystems that exploit environmental cues, like pH or enzymes, can trigger controlled proteins and peptides therapeutics release within brain tissue, optimizing therapeutic outcomes (Farkhani et al., 2014) (Qin et al., 2019).
3.1 Protein therapeutics in various neurological disorders

3.1.1 Alzheimer’s disease
Alzheimer’s disease (AD) is driven by abnormal protein aggregation. Amyloid-β (Aβ) peptides aggregate to form plaques outside neurons, while hyperphosphorylated tau proteins aggregate into neurofibrillary tangles inside neurons. Moreover, these aggregates disrupt neuronal communication and trigger inflammation, contributing to cognitive deterioration in AD patients (Muralidhar et al., 2020). Nano-enabled protein therapeutics target AD by binding to Aβ plaques, preventing their aggregation and subsequent neural damage. Furthermore, certain proteins can modulate tau phosphorylation, mitigating neurofibrillary tangle formation. In addition to this, protein-based neurotropic factors foster cognitive function recovery (Baranowska et al., 2020).

3.1.2 Parkinson’s disease
Parkinson's disease (PD), a neurodegenerative disorder, is associated with the degeneration of neurons that produce dopamine in the substantia nigra region of the brain. This results in disrupted signaling with the basal ganglia, leading to bradykinesia, tremors. Moreover, the accumulation of α-synuclein protein in Lewy bodies contributes to neuronal toxicity and dysfunction in PD (Raj et al., 2021). By delivering specific protein therapeutics such as brain-derived neurotropic factor, aimed to counteract the degeneration of dopaminergic neurons in the substantia nigra and heralding a promising avenue for more effective and tailored treatments (Schüle et al., 2009).

3.1.3 Huntington’s Disease
The presence of mutant huntingtin protein aggregates within neurons, leading to neurodegeneration primarily in the striatum and cortex leading to Huntington’s disease (HD). HD is associated with imbalances in neurotransmitter systems, particularly dopamine, resulting in motor dysfunction and cognitive impairment. Nanocarrier-enabled protein therapeutics can be designed to reduce the production of mutant huntingtin or enhance its clearance (Vonsattel et al., 1998).

3.1.4 Amyotrophic Lateral Sclerosis
Amyotrophic Lateral Sclerosis (ALS) is characterized by motor neuron degradation. Protein therapeutics targeting ALS often focus on regulating misfolded proteins like SOD1 or TPD-43.
Moreover, by upregulating protein clearance mechanisms, these therapies aimed to reduce toxic protein aggregates in neurons (Blokhuis et al., 2013).

3.1.5 Multiple Sclerosis
Multiple Sclerosis (MS) is a chronic autoimmune disease that affects the CNS. The immune system mistakenly attacks the protective covering of nerve fiber called myelin, leading to inflammation and loss of myelin. By targeting specific proteins involved in the immune response, these therapies can modulate immune activity. For instance, monoclonal antibodies can bind to surface markers like CD20 on B cells, downregulating their numbers (Del Gatto et al., 2021).

3.1.6 Neuropathic pain
Neuropathic pain is a type of chronic pain often described as burning, shooting, and electric-like shock. It can be challenging to treat, requiring a comprehensive approach that is nanocarrier-enabled protein therapeutics for the treatment of neuropathic pain. By modulating neurotransmitter release and receptor activation, protein therapeutics can mitigate the hyperexcitability of neurons in the pain pathway. In addition to this, these protein therapeutics upregulate the production of endogenous analgesic agents such as enkephalins, to dampen pain signals (Cavalli et al., 2021).

3.1.7 Epilepsy
Epilepsy is a neurological disorder characterized by recurrent unpredictable seizures. For its management protein therapeutics for epilepsy target the molecular intricacies of neural circuits. The therapeutic proteins can modulate neurotransmitter release, ion channel activity and restore balance in aberrant neuronal networks. Additionally, by regulating synaptic transmission, protein therapeutics help prevent hyperexcitability (Stafstrom et al., 2006).

3.2 Peptide therapeutics in various neurological disorders
3.2.1 Alzheimer’s disease
AD is characterized by the accumulation of amyloid-beta (Aβ) plaques and tau tangles in the brain, leading to cognitive decline and neuronal damage. Nano-enabled brain delivery of peptides therapeutic holds significant promise in the treatment of AD. Moreover, this short chain of amino acids can be designed to interfere with the key molecular processes underlying the
disease. For instance, certain peptides are engineered to bind to and inhibit the aggregation of Aβ proteins, preventing the formation of toxic plaques in the brain. Other peptides, might target tau protein aggregation, reducing the formation of neurofibrillary tangles. By specifically addressing these pathological features, peptide therapeutics hold the potential to slow down the progression of AD. As the field of peptide therapeutics research continues to advance, these tailored molecules provide a promising avenue for developing more effective and less invasive treatments for AD (Muralidhar et al., 2020) (Baranowska et al., 2020).

3.2.2 Parkinson’s disease

Peptide therapeutics offer a promising avenue for treating PD. One notable approach involves targeting alpha-synuclein, a protein implicated in the formation of Lewy bodies, the pathological hallmark of PD. Additionally, peptide therapeutics can modulate neurotransmitter systems, such as dopamine, whose deficiency leads to motor impairments in PD. By upregulating dopamine release, regulating receptor activity, or promoting neuron survival, peptide therapeutics hold the potential to alleviate motor symptoms and slow disease progression. Nanocarriers also provide sustained and controlled release of peptides, maintaining therapeutic levels over an extended period. Furthermore, nanocarriers protect peptide therapeutics from enzymatic degradation and upregulate their stability, bioavailability, and efficacy (Schüle et al., 2009).

3.2.3. Seizure

Peptide therapeutics offer a promising avenue for the management of seizures through their targeted modulation of specific molecular pathways involved in epileptic activity. Certainly, peptides can act as antagonists or agonists to neurotransmitter receptors, such as GABA receptors. GABA is a major inhibitory neurotransmitter that plays a crucial role in dampening excessive neuronal firing. Peptides designed to enhance GABAergic transmission can promote inhibitory signaling, reducing neuronal hyperexcitability and the likelihood of seizure initiation (Clynen et al., 2014). Moreover, nanocarriers such as liposomes or polymeric nanoparticles, serve as delivery vehicles for these peptides. First, the nanocarrier protects the peptides from enzymatic degradation, enhances the stability, and can target ion channels implicated in seizure generation, like sodium and calcium channels. By modulating these channels, peptides can regulate the flow of ions across neuronal membranes, controlling neuronal excitability and preventing the rapid firing characteristics of seizures. Furthermore, some peptides possess anti-
inflammatory and neuroprotective properties. Seizures often lead to neuroinflammation and oxidative stress, contributing to neuronal damage (Lima et al., 2022).

3.2.4. Autism

Autism spectrum disorder (ASD) is characterized by complex neural dysregulation, and targeting neurochemical imbalances and signaling pathways could alleviate symptoms. Oxytocin and vasopressin analogs, for instance, aim to modulate social behavior and communication deficits, core features of ASD. Moreover, neuropeptide Y has been explored for its anxiolytic and stress-reducing effects, addressing anxiety often accompanying ASD. Furthermore, targeting glutamatergic and GABAergic systems using specific peptides could help regulate excitatory-inhibitory balance, potentially ameliorating sensory sensitivities and repetitive behaviors. Nanocarriers enhance peptide delivery to the brain by crossing the BBB, ensuring efficient access to neural circuits. Encapsulation protects peptides from degradation and sustains therapeutic effects. Additionally, targeted delivery can modulate neurotransmitter imbalances and restore synaptic homeostasis (Blokhuis et al., 2013).

3.2.5. Neuropathic pain

Peptide therapeutics offer a promising path for treating neuropathic pain by targeting specific mechanisms involved in pain signaling. These nano-enabled delivery systems facilitate targeted drug delivery to the CNS, where they can modulate key pain pathways. Moreover, these peptides often mimic endogenous signaling molecules and can modulate pain pathways with high specificity. For instance, some peptides target neuropeptide receptors to regulate neurotransmitter release, altering pain transmission. Furthermore, others inhibit ion channels associated with pain signaling, dampening aberrant pain signals. Additionally, peptide therapeutics can influence inflammatory processes that contribute to neuropathic pain, helping to reduce tissue damage and sensitization. In addition to this, by targeting key players in pain pathways, these therapies offer a focused and potentially safer alternative to other conventional treatments (Cavalli et al., 2019).
4. Nano-enabled brain delivery of proteins and peptides therapeutics in various neurological disorders

4.1 Nanocarriers enabled delivery of protein therapeutics in various neurological disorders

4.1.1 Alzheimer’s disease

The aggregation and accumulation of Aβ peptide are widely believed to be the primary cause of AD pathogenesis. To address this issue, Jeon and their colleagues conducted a study on the therapeutic effects of Vit D-BP-loaded PLGA NPs in Aβ overexpressing 5XFAD mice. They also examined the attributes of Vit D-BP-PLGA NPs. Using thioflavin-T assay, *in vitro* studies revealed that Vit D-BP-PLGA NPs had a significant inhibitory effect on Aβ aggregation. Moreover, *in vivo* studies on 5XFAD mice demonstrated that intravenously administered Vit D-BP-PLGA NPs remarkably reduced Aβ accumulation, neuroinflammation, cognitive dysfunction, and neuronal loss. Therefore, it can be deduced that Vit D-BP-PLGA NPs hold immense potential as a therapeutic option for treating AD (Jeon et al., 2019). In another study researchers have devised a method for administering drugs via macrophage (MA) membrane-coated solid lipid nanoparticles (SLNs) that have been tailored with rabies virus glycoprotein (RVG29) and triphenylphosphine (TPP) molecules. The objective of this modification is to facilitate efficient antioxidant delivery to neuronal mitochondria. The researchers found that the MA membrane coating camouflaged the SLNs from being eliminated by organs that are rich in the reticuloendothelial system (RES) by inheriting the immunological characteristics of macrophages. Additionally, the drug delivery system exhibited the ability to cross the blood-brain barrier (BBB) and selectively target neurons when decorated with RVG29 on the surface. The researchers also discovered that genistein (GS) encapsulated in the RVG29/TPP-MA-SLNs-GS exhibited the most favorable effect on relieving AD symptoms both *in vitro* and *in vivo* (Han et al., 2021). In a groundbreaking study, first-time scientists explored the potential of protein-capped (PC) iron oxide (Fe$_3$O$_4$) and CdS metal nanoparticles to inhibit Tau aggregation *in vitro*, to manage AD. To accomplish this objective, the authors employed fluorescence spectrometry, sodium dodecyl sulfate-polyacrylamide gel electrophoresis, and electron microscopy. Interestingly, the PC-Fe$_3$O$_4$ nanoparticles that were produced biologically did not have any detrimental effects on the survival of neuroblastoma cells. Additionally, the PC-CdS nanoparticles displayed dual properties of inhibiting and disaggregating Tau. These findings offer a novel approach to designing anti-Tau aggregation cargo and provide valuable insights.
into the role of biological nanostructures in AD, as well as their potential as drug delivery vehicles (Sonawane et al., 2019).

4.1.2 Parkinson’s disease

Huang and his team have devised a gene delivery mechanism that employs angiopep-conjugated dendrigraft poly-L-Lysin (DGL) to examine its neuroprotective qualities in a chronic form of Parkinson's disease brought about by rotenone. Angiopep, which is a ligand, attaches exclusively to the low-density lipoprotein receptor-related protein (LRP), which is overexpressed on the blood-brain barrier (BBB) and is subsequently combined with biodegradable DGL via hydrophilic PEG, resulting in DGL-PLGA-angiopep. DGL-PLGA-angiopep was produced by the authors making use of 1H-NMR. DGL-PLGA-angiopep/hGDNF nanoparticles, once they encapsulated the therapeutic gene human glial cell line-derived neurotrophic factor (hGDNF), had a spherical shape and a particle size of 119 ± 12 nm, as well as a ZP of 8.2 ± 0.7 mV. Moreover, the produced nanoparticles exhibited greater cellular uptake and gene expression in brain cells in comparison to their unaltered counterparts. In vivo findings indicated that rats that were given DGL-PLGA-angiopep/hGDNF nanoparticles demonstrated improved locomotor activity and the recovery of dopaminergic neurons, eclipsing the results of other groups (Huang et al., 2013).

4.1.3 Huntington’s Disease

Previous research studies have shown that the brains of HD disease patients have insufficient levels of selenium (Se) and selenoproteins. However, there is potential to reduce neuronal loss and dysfunction by improving Se homeostasis in the brain. A team of researchers conducted a study utilizing Se nanoparticles for HD therapy, which regulated HD-related neurodegeneration and cognitive decline in transgenic HD models of Caenorhabditis elegans (C. elegans). Additionally, the authors discovered that Se nanoparticles reduced oxidative stress, inhibited the aggregation of huntingtin proteins, and decreased the expression of histone deacetylase family members at mRNA levels. The findings imply that Se nanoparticles are a promising option for HD therapy (Cong et al., 2019). In a distinct investigation, Debnath and colleagues produced nanoparticles from poly(trehalose) which can obstruct amyloid/polyglutamine aggregation in both intracellular and extracellular environments, thereby reducing cytotoxicity and preventing polyglutamine aggregation in the HD B6CBA-Tg (HDexon1) 62Gpb/3J mouse model. These
nanoparticles have a size of approximately 20-30 nm and are composed of a 6 nm iron oxide core and a zwitterionic polymer shell that contains about 5-12 wt. % covalently linked trehalose. The authors’ data confirmed that these developed nanoparticles are 1000 times more effective than molecular trehalose in preventing protein fibrillation in the extracellular environment, hindering the aggregation of polyglutamine containing mutant huntingtin protein in model neuronal cells, and suppressing mutant huntingtin aggregates in HD mouse brains. Moreover, the researchers have demonstrated that a zwitterionic surface charge and a trehalose multivalency of around 80-200 are crucial for effective brain targeting, entry into neuronal cells, and suppression of mutant huntingtin aggregation in nanoparticle form. Hence, this technique can be extended to \textit{in vivo} applications as a way of combating protein-aggregation-derived HD (Debnath et al., 2017).

4.1.4 Amyotrophic Lateral Sclerosis

ALS is a disease that is fatal and leads to the gradual destruction of motor neurons. In a study previously published by Vimal et al., it was demonstrated that motor processivity could be restored through the use of gold nano-chaperon treatment \textit{in vitro}, but this was hindered by the addition of the A4V SOD1 transgenic mice model of ALS. Currently, a team of authors has evaluated the pharmacological potential of gold (Au) PEG nanoconjugate in transgenic SOD1G93A mice \textit{in vivo}. In addition, the authors found discrepancies in the detection of symptom onset and ALS progression with Au-PEG treatment, as indicated by behavioral tests such as rotarod and walking tests. Furthermore, gastrocnemius muscle histopathology showed a significant alteration with treatment. Molecular docking studies also revealed that the addition of A4V SOD1 mutant to the tubulin dimer negatively impacted the binding of the kinesin motor to tubulin $K_d = 1.4 \times 10^{-7} \text{ mol L}^{-1}$ vs $K_d = 2.5 \times 10^{-9} \text{ mol L}^{-1}$, while the exogenous addition of Au nanoparticles to the protein complex of A4V SOD1-tubulin significantly increased the binding affinity of kinesin to the tubulin dimer $K_d = 2.7 \times 10^{-12} \text{ mol L}^{-1}$. Therefore, the use of Au nanoparticles could be promising in the treatment of the complex disease ALS (Vimal et al., 2022). Recent research in neurobiology and neurodegenerative diseases has sparked increasing interest in exosomes and their capacity to transport active molecules to reprogram recipient cells. Changes in the content of exosomal proteins and nucleic acid profiles found in human biological fluids have been linked with ALS. In addition, in this study, the authors studied the potential
therapeutic benefits of exosomes for delivering exosomal proteins in the treatment of ALS by exploring the rapid development of using nanoparticles for cargo delivery (Chen et al., 2021).

4.1.5 Multiple Sclerosis

MS is a disease of the CNS that is both autoimmune and inflammatory. Research has shown that autoantibodies react with components of the myelin sheath, including MOG. In this unique study, researchers analyzed MOG peptide sequences similar to the HERV-W protein, which is a family of retroviruses found in humans. The study also employed atomic force spectroscopy and silver nanoparticles to investigate the potential for molecular mimicry between anti-HERV-W antibodies and MOG epitopes. The authors of the study also discovered molecular recognition between the anti-HERV-W antibody and both HERV-W and MOG epitopes using these approaches. Further analysis showed that the force curves for HERV-W and MOG peptides had specific non-linear shapes, and the median adhesion force values for antigen-antibody interaction were within the expected range, at 163 pN and 178 pN, respectively (de Luca et al., 2019).

4.1.6 Neuropathic pain

Lalani and colleagues endeavored to create surface-engineered nanoparticles of Lamotrigine (LTG) through nanoprecipitation, utilizing transferrin and lactoferrin as ligands to deliver a higher amount of cargo. Furthermore, the nanoparticles were functionalized with lactoferrin and transferrin on the surface. The produced nanoparticles were evaluated for various physicochemical parameters and stability. In vivo, biodistribution demonstrated a preference for brain targeting and reduced accumulation in non-target organs over an extended period. Additionally, a pharmacodynamic study in a nerve injury mice model confirmed that the approach used for LTG can help upregulate the clinical applications of LTG due to brain targeting and reduced side effects (Lalani et al., 2015). In a separate research study, scientists developed nanoparticles that were loaded with p38 siRNA. They then investigated whether these nanoparticles could alleviate neuropathic pain in rats that had undergone spinal nerve ligation. Specifically, the scientists wanted to see if the nanoparticles could suppress spinal microglia activation via p38 targeting. To fabricate the nanoparticles, the authors used a sonication method and measured particle size and zeta potential for physical characterization. The p38 siRNA nanoparticles were administered intrathecally into rats with spinal nerve ligation to assess their impact on pain behavior. The scientists found that the nanoparticles led to a reduction in
mechanical allodynia and microgliosis in the spinal dorsal horns of the rats. Moreover, the p38-related proinflammatory mediators were downregulated. These findings suggest that p38 in the spinal microglia is an important factor in treating neuropathic pain (Shin et al., 2018).

4.1.7 Epilepsy
The neuropathology of temporal lobe epilepsy (TLE) is characterized by brain inflammation, specifically the activation of interleukin-1β (IL-1β) induced by activated glial cells, which has been identified as a novel mechanistic target for treatment. Fu et al. conducted a study to determine the feasibility of using superparamagnetic iron oxide nanoparticles (SPIONs) with anti-IL-1β monoclonal antibody attached to provide MRI diagnosis and targeted therapy for the neutralization of IL-1β overexpressed in the epileptogenic zone of an acute rat model of TLE. The study utilized in vivo lithium-chloride pilocarpine-induced TLE models, and Western blot, Perl's iron staining, and immunofluorescent double label staining were performed following an MRI examination. Additionally, magnetic IL-1β monoclonal antibody-SPIONs were administered intravenously and were concentrated in the astrocytes and neurons in epileptogenic tissues, rendering these tissues visible on MRI while simultaneously delivering anti-IL-1β monoclonal antibody to the epileptogenic focus (Fu et al., 2016).

4.2 Nanocarriers enabled delivery of peptide therapeutics in various neurological disorders

4.2.1 Alzheimer’s Disease
For improved brain uptake of NAPVSIPQ, a neuropeptide fragment via intranasal delivery to treat Alzheimer’s disease, Liu and coworkers aimed to study lactoferrin conjugated PEG-PCL nanoparticles, (Lf@PEG-PCL NPs). The authors performed a comparison between modified and unmodified nanoparticles and concluded that Lf@PEG-PCL NPs strongly increased cell accumulation in 16HBE14o cells through direct translocation as well as caveolae/clathrin-mediated endocytosis. The distribution of coumarin-6 in the brain was then superior to that of unmodified nanoparticles. In addition, the researchers of this work used the NAPVSIPQ peptide as a model medication and noted the neuroprotective and memory-enhancing effects of Lf@PEG-PCL even at lower doses, which was supported by behavioral, biochemical and histopathological data. According to the results of this intriguing study, Lf@PEG-PCL NPs hold a huge potential to deliver the peptides and proteins in the brain (Liu et al., 2013). Using a different approach, another group of researchers prepared nanogels based on poly(N-
vinylpyrrolidone) and conjugated with insulin (NG-In) for intranasal administration for the
treatment of Alzheimer's disease. The authors concluded that urine clearance occurs within 24
hours after administration. In addition, no immune response was elicited by the injection of NG
and no morphological changes occurred in the tissues. Furthermore, no change in the nasal
epithelium was seen after nasal delivery of the NG-In to the brain, suggesting that the
formulation is biocompatible and safe for the male C57BL/6J (B6) mice in vivo. It was also
shown that free insulin administration improved the distribution of MG-In to the various sections
of the brain as indicated by the level of Akt activation. The study claimed that synthetic NG-In
increases brain insulin uptake through nasal delivery to the brain and strongly stimulated further
research on its potential as a drug delivery carrier for improved brain disease treatment (Picone et
al., 2018).

4.2.2 Parkinson's Disease

In rat and primate models of PD, all neurotrophic gene therapy strategies have been successful
and completely benign, although some of them, particularly those containing GDNF, cause
inappropriate behavioral problems due to abnormal innervation of regrowing dopaminergic
fibers and a reduction in dopamine polymerization. The selectivity of the endocytic mechanism
of the NTS-NTSR1-mediated polyplex to deliver the transgene into intact dopamine neurons is
an attractive feature in the management of neurological diseases (Martinez et al., 2012).

For improved penetrability of proteins or pharmaceutical drugs in the CNS, many targeting
ligands have been used, including Tet1, lactoferrin, peptide TGN and rabies virus glycoprotein
(RVG), etc. The fact that cell-penetrating peptides such as Arg-9, penetratin, and TAT can be
intrinsically neuroprotective and provide an additional benefit to functionalized vectors while
masking the source of the benefits. While anti-glioblastoma RGD-modified polymeric micelles
have recently enabled highly efficient delivery to the brain, PD therapies may potentially require
precise targeting to the striatum or the dopaminergic neurons themselves to overcome off-target
effects (Newland et al., 2015) (Ozkizilcik et al., 2019) (Karthivashan et al., 2020).

An evasive liposome containing the dopamine (DA) raw material N-3,4-bis(pivaloyloxy)-
DA (BPD) was prepared some time ago by a research team and complexed using the RVG29
peptide as the targeting moiety. These particles showed better interaction with dopaminergic
cells and ACh receptors BPD RVG29 liposomes were successfully passed through after
intravenous administration transported the BBB as significant amounts penetrated in the brain,
and the treatment may have reduced the PD-like molecular impairments observed in an animal model with unilateral 6-OHDA lesions (Qu et al., 2018). Interestingly, transcription peptide transactivator complexed chitosan coated GDNF-impregnated NLCs were studied for brain localization. Particle size analysis data showed the nanosize range with a narrow PDI with good entrapment efficiency. Upon administered intranasally to the PD-like animal model, these particles increased the amount of tyrosine hydroxylase-positive fibers in STN and SNpc and reduced behavioral disturbances compared to the free form considerably (Hernando et al., 2018).

Gao and team explored $^{125}$I-vasoactive intestinal peptide (VIP) loaded wheat germ agglutinin (WGA) modified PLGA nanoparticles via intranasal delivery for CNS delivery. In vivo studies showed that intact $^{125}$I-vasoactive intestinal peptide from the plain and modified nanoparticles was increased by five folds and eight folds, respectively, vis-à-vis $^{125}$I-VIP solution in the mice brain following intranasal delivery. There was improvement in the spatial memory of the animals after unmodified and WGA modified nanoparticles. Additionally, biodistribution profiles of the WGA nanoparticles showed better interaction with olfactory mucosa in comparison to oral respiratory zone. According to the results, it is suggested that wheat germ agglutinin tailored nanoparticles could be a promising approach for biological drugs such as protein and peptides (Gao et al., 2007).

In a study, N-3,4-bis(pivaloyloxy)-dopamine-derived nanostructured liposomal and a brain-targeted distribution network of twenty-nine amino acid peptides (RVG29) made up of rabies virus glycoprotein were examined. Both endothelial and dopaminergic cells showed markedly improved cellular absorption with better BBB invasion. Because the RVG29 LNPs were specifically targeted to the substantia nigra and striatum, there was also an improvement in clinical efficacy (Qu et al., 2018). Zhao and team aimed to study neuropeptide Substance P (SP) gelatin nanoparticles (GNPs) against animal model of Parkinson’s disease. Cellular studies on PC-12 cells exhibited biocompatible nature and suppression of cell apoptosis from the GNPs vis-à-vis its free counterpart. Improved behavior and enhanced neuron regeneration were also observed in behavioral assessment of hemiparkinsonian rats, supporting the idea that SP might be specifically delivered to the brain via generation of its BNPs (Lu et al., 2015).

4.2.3 Seizure

Kubek et al investigated thyrotropin-releasing hormone (TRH), loaded polylactide nanoparticles (PLA-NPs) in the animal model of epilepsy and in humans. In this study, they found
concentration-dependent effects on behavioral stages one to four followed by stage five epilepsies. In vitro cellular uptake studies on hippocampal neurons exhibited better uptake and transport of physically mixed dye and dye-conjugated nanoparticles via endocytosis process. In vivo studies using fluorescent dye loaded nanoparticles on Sprague-Dawley rats demonstrated availability of nanoparticles in the rat’s brain following intranasal delivery. Authors concluded that much work is needed to establish the mechanism of PLA-NPs uptake in the olfactory neuroepithelium (Kubek et al., 2009). In another research, oxytocin loaded nanoparticles were explored to increase resistance to seizure and improve social behaviour via intracerebral administration. In vivo studies on male CF1 mice demonstrated protective effect of nano-oxytocin against induced seizure. Social behaviour studies also displayed improvement in the sociability and social interaction of the RH/+ mutants mice dosed with nanoparticle formulation in comparison to oxytocin solution. Authors concluded the seizure protective nature of the nanoparticle could be due to oxytocin receptors in the RH/+ mutants, an animal model of Scn1a-derived epilepsy (Wong et al., 2021). In a similar study by Sahin and group, oxytocin encapsulated albumin nanoparticles (OXT- NPs) were investigated in terms of cognition functions, genesis of neuron and cell damage via intranasal delivery. According to the in vivo studies, OXT-NPs were able to prevent kindling development and showed promising effects on epilepsy severity. Additionally, histological analysis revealed the protective nature of OXT-NPs against the Pentylenetetrazole-induced damage to the hippocampal neurons. Another analysis showed increased neurogenesis and decreased apoptosis in the hippocampal region following OXT-NPs. Hence, authors concluded that OXT-NPs may have a positive effect on the Pentylenetetrazole-induced epilepsy, however more research is needed to examine total antioxidant capacity and total oxidant capacity in the serum (Sahin et al., 2022).

4.2.4 Autism

OXT has immense application as a therapeutic to treat the brain disorders such as autism, however frequent dosing, metabolism in the blood and brain penetration limits its further use. In this regard, Zaman et al attempted to prepare PLGA and or bovine serum albumin (BSA) nanoparticles conjugated with either transferrin (Tf) or rabies virus glycoprotein (RVG) as targeting moiety. The prepared nanoparticles were subjected to analyze particle size, surface charge, drug loading and release profile. The authors found that the particles carrying negative zeta potential with particle size range between 100 to 278nm and encapsulation efficiency up to
75%. There was faster release of OXT initially from the BSA formulations vis-à-vis PLGA particles and sustained release later on. Overall, authors concluded that RVG-conjugated BSA formulations showed a better carrier system for brain delivery of oxytocin (Zaman et al., 2018). By the same group, OXT loaded Tf and BSA conjugated nanoparticles were explored in artificial BBB model and in vivo brain transport. According to the results, they found no significant effect on uptake of developed nanoparticles irrespective (absence and presence) of the cellular barrier. Additionally, in vivo studies on mice revealed higher accumulation of OXT in the blood and CSF. Interestingly, peptide loaded nanoparticles showed better pro-social effects compared to oxytocin solution. Hence, it could be concluded that nano-encapsulated OXT formulation holds a greater potential in the animal model of autism spectrum disorders (Oppong et al., 2019).

4.2.5 Neuropathic pain
In a study, Leucine enkephalin (Leu-Enk), a peptide encapsulated with methyl modified chitosan derivative (TMC) nanoparticles were prepared using ionic gelation method. Drug loading experiments showed a good loading capacity (14.1.3%) and peptide encapsulation efficiency (78.28%). The authors also noted a 35-fold increase in the amount of Leu-Enk peptide released from nanoparticles from nasal mucosa compared to Leu-Enk solution, and they confirmed a remarkable permeability coefficient of the charged peptide through porcine nasal mucosa. In vivo studies in mice via intranasal delivery revealed greater concentration of fluorescent marker NBD-F-labeled Leu-Enk. Finally, hot plate and acetic acid method showed a markedly improved antinociceptive effect of Leu-Enk (Amidi et al., 2006).

4.2.6 Miscellaneous
A growing number of studies in recent years have addressed drug and gene delivery using a mixture of nanomaterials and cell-penetrating peptides (CPPs). This codelivery produces a potent therapeutic effect in vitro and in vivo. In this regard, Yuan and team investigated doxorubicin loaded liposomal formulation anchored with octaarginine (DOX@R8PLP). After treatment at 3.6 M for 24 h, R8PLP absorption by U87-MG cells increased 8.6-fold compared to liposomes that had not been treated, and cell viability decreased by 16.18%. Brain biodistribution was significantly improved and the area under curve of DOX@R8PLP was 2.4-fold greater vis-a-vis untreated formulation (Zhang et al., 2021). A lectin-like peptide called odorranalectin was first discovered in epidermal secretions. Urocortin peptide (UCN) was nasally delivered to the brain
by Wen and co-workers using odoranalectin. In addition, odoranalectin was linked to PEG-PLGA to reduce its mutagenicity and toxicity. As a result, odoranalectin can be used as a potentially evolving peptide for delivering therapeutics at a specific site (Nicolas et al., 2013).

In another study, Migone and colleagues prepared neuropeptide dalargin (DAL)-treated nanoparticles by synthesizing a quaternary ammonium derivative of chitosan coupled with methylcyclodextrin (DAL-NP). The particle size was shown to be 227.7 nm, Z.P. was +8.60 mV, and the encapsulation efficiency was 89% during the characterization research. In addition, DAL-NP could be safely used on CaCo-2 or bEnd.3 cells, the latter being used as a BBB model as shown in the Fig. 3. The developed nanoparticles could be uptake by these cells via transepithelial pathway. Authors concluded that orally ingested dose of DAL could be transported to the brain by DAL-NP, however further research is required to confirm these results (Migone et al., 2020).

In another research, insulin loaded PLGA and chitosan coated solid lipid nanoparticles (CHT-SLNs) were designed and evaluated. The prepared nanoparticles showed favorable physical and chemical properties that supported intranasal application. In vitro studies showed that chitosan-coated SLNs were superior to native insulin in terms of nasal dispersion, mucosal adhesion, and drug release rate. In addition, research on human nasal epithelium and brain endothelial cell lines demonstrated the safety of intranasal application of nanoparticles as shown in the Fig. 4. Moreover, CHT-SLNs displayed higher cellular permeability across the nasal epithelium compared to PLGA nanoparticles. Hence, it is suggested that CHT-SLNs might help ensure structural stability, improve nasal absorption, and then provide prolonged drug release (Akel et al., 2021).

5. Conclusion and Future Perspective

It could be concluded that in the coming time many current organic-based formulations will be replaced by peptide- and protein-based pharmacological delivery to the brain, which is rapidly emerging as a very important class of therapeutics. Nevertheless, the BCSF barrier and BBB are the best examples of biochemical dynamic barriers that often limit the availability of effective non-invasive treatments for neurological disorders. Many therapeutics such as protein and peptidal, anticancer and numerous brains targeted neuron protective peptides are insurmountably hindered by BBB, consequently, presents a huge challenge to delivery these at the local site of action. Therefore, targeting BBB and transporting the therapeutics inside the brain requires
crossdisciplinarity research in term of drug delivery sciences and technology, neuroscience, and biological sciences. Therefore, one of the greatest concerns today is the treatment of neurological diseases. Various methods have been employed to circumvent the limitations of proteins and peptides as a result of technological and formulation advances. There are numerous proteins and peptides encapsulated carrier systems based on nanotechnology to deliver the drugs in brain such as prolease technology, nano/microparticles of peptides. These systems proved to be excellent methods of protein and peptide delivery. Peptidal drugs gained huge step forward with advancement in the scientific research incorporating new disease targets, employing newer chemical techniques to intensify diversity, linker strategies, and developing improved biopharmaceutical properties. Lately, a lot of nanomedicines has been attempted to deliver CNS therapeutics, including polymeric nanoparticles, SLNs, liposomes, micelles, dendrimers, and nanosuspensions. New targeting molecules, improved BBB permeability and reduced neurotoxicity should be the main goals of CNS nanomedicine research in the future. Eventually, new strategies for the formulation, delivery, and improved mean residence time of peptide drugs will expand the uses of this extraordinary therapeutics. The comparative effectiveness of the nose to brain in comparison to rest of the routes of administrations has not yet been demonstrated in clinical trials of direct intranasal delivery and devices. For improved brain delivery of therapeutics via nose to brain targeting, attention must be paid to both clinical studies and preclinical studies. In addition, detailed evaluations of toxicodynamic studies of drugs and excipients as well as the nanotoxicity of nanocarriers are required. Researchers must also consider the safety and biocompatibility of the DDS, host and material toxicity, tissue-specific effects, cost effectiveness and avoiding cost barriers, biodegradability, etc. If they want to create more effective protein and peptide DDS. Although most studies with cell-permeable peptidal therapeutics and pH-sensitive pharmaceutical nanocarriers are still in the pipeline, it is anticipated that the introduction of newer drugs and therapeutic regimens very soon. We hope that research will continue to uncover new protein-peptide delivery options for the brain.

**Authors contribution:** Arora Sanchit: Wrote or contributed to the writing of the manuscript; Bajaj Tania: Wrote or contributed to the writing of the manuscript; Kumar Jayant: Wrote or contributed to the writing of the manuscript; Goyal Manoj: Wrote or contributed to the writing of
the manuscript, Singh Arti: Participated in manuscript design and Edited the manuscript; Singh Charan: Supervision, Participated in manuscript design and Edited the manuscript

Conflict of interest: None declared

Funding details: None

Acknowledgment: Authors duly acknowledge HNB Garhwal University (A Central University), Srinagar Gharwal, Uttarakhand for providing basic infrastructure to compile this work.

Data Availability: This article contains no datasets generated or analyzed during the current study.
References:


Chen Y, Huang L, Qi X and Chen C (2019) Insulin Receptor Trafficking: Consequences for Insulin Sensitivity and Diabetes. **20**.


Rhea EM and Rask-Madsen C (2018) Insulin transport across the blood-brain barrier can occur independently of the insulin receptor. 596:4753-4765.


Song J and Lu C (2021) Design and Development of Nanomaterial-Based Drug Carriers to Overcome the Blood-Brain Barrier by Using Different Transport Mechanisms. 22.


Figure Captions

Figure 1 Mechanisms for the absorption of high molecular weight therapeutics across the BBB. These includes the transcellular lipophilic pathway enables lipophilic molecules to pass through the endothelial cells’ lipid membranes. Secondly, carrier mediated transport utilizes specialized proteins within endothelial cell membranes to facilitate the passage of specific molecules. Paracellular transport allows molecules to move through the spaces between endothelial cells, regulated by tight junction. Additionally, AMT relies on the interaction between charged molecules and charged surfaces of endothelial cells, aiding in the transport of charged substances. These processes work synergistically to enable the absorption of high molecular therapeutics into brain tissue, overcoming the barrier presented by BBB.

Figure 2 Receptor-mediated targets (RMT) for transport at the BBB. Herein, it shows major specific receptors expressed on the luminal surface on brain endothelial cells, including transferrin receptor (TfR), low density lipoprotein, receptors (LDLR), and insulin receptors (IR), and others receptors. Each receptor serves as a key player in mediating the transport of respective ligands from the bloodstream into the brain parenchyma. Additionally, these receptors selectively recognizes and binding to various ligands, including hormones, nutrients, and specific therapeutic agents.

Figure 3 Confocal fluorescence micrograph panel for bEnd.3 monolayers used for permeation studies with FITC-labelled DAL-NP at incubation times of 1, 2, and 3h. Single acquisitions of channels: blue for Hoechst-labeled cell nuclei, red for TetramethylRhodamine-labelled actin filaments (TRICT), green for the internalized NP (ImageJ software, National Institutes of Health, Bethesda, Maryland, USA). Intriguingly, a higher portion of residual DAL was observed in the apical chamber, even with equal incubation times compared to Caco-2 cells. This suggests that Caco-2 cells possess a notably robust and intricate enzyme system, rendering them more proficient in peptide degradation that bEnd.3 cells, despite the latter heightened sensitivity as revealed by cytotoxicity tests. The hypothesis of DAL permeating via the transcellular route in bEnd.3 cells finds support in confocal microscopy images, vividly displaying the internalization of FITC labeled DAL-NP within the cells and deduced that the cell monolayer and morphology were not altered after comparing with bEnd.3 control. Reprinted from Shin J, Yin Y, Park H, Park S, Triantafillu UL, Kim Y, Kim SR, Lee SY, Kim DK, Hong J and Kim DW (2018) p38 siRNA-encapsulated PLGA nanoparticles alleviate neuropathic pain behavior in rats by

This article has not been copyedited and formatted. The final version may differ from this version.
inhibiting microglia activation. Nanomedicine 13:1607-1621 Copyright: © 2020 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CCBY) license (https://creativecommons.org/licenses/by/4.0/).

**Figure 4** Cell viability of RPMI 2650 nasal epithelial cells after the treatment with insulin, insulin NPs, and HCl measured by impedance. The kinetic curve of cell viability during the 20-h treatment. It illustrates the comparable kinetics of the nanoparticles (NPs) in the epithelial and endothelial models when compared to the untreated control group. It is worth noting that in hCMEC/D3 cells, both insulin PLGA-NPs and insulin C-PGA-NPs exhibited a slight decrease in cell index values. However, these values consistently remained within the non-toxic range (above 0.75). The significant differences observed at the 1-hour time point for both cell types (Figures 4. B and D) can be attributed to an extremely low standard deviation, rather than a toxic effect of the treatments. Neither the RPMI 2650 nasal epithelial cells nor the hCMEC/D3 endothelial cells displayed any noticeable cell damage following treatment with insulin and insulin-containing NPs. Values are presented as means ± SD, n = 6–12 Statistical analysis: ANOVA followed by Dunett’s test. TX-100: Triton X-100. * p < 0.05, ** p < 0.01 compared with the control. Reprinted from references (78) Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https://creativecommons.org/licenses/by/4.0/).
Figure 1