# Precision Nanomedicine in Neurodegenerative Diseases

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ABSTRACT The treatment of neurodegenerative diseases remains a tremendous challenge due to the limited access of molecules across the blood—brain barrier, especially large molecules such as peptides and proteins. As a result, at most, a small percentage of a drug that is administered systemically will reach the central nervous system in its active form. Currently, research in the field focuses on developing safer and more effective approaches to deliver peptides and proteins into the central nervous system. Multiple strategies have been developed for this purpose. However, noninvasive approaches, such as nanostructured protein delivery carriers and intranasal administration, seem to be the most promising strategies for the treatment of chronic diseases, which require long-term interventions. These approaches are both target-specific and able to rapidly bypass the blood—brain barrier. In this Perspective, we detail some of these strategies and discuss some of the potential pitfalls and opportunities in this field. The next generation strategies will most likely be more cell-type-specific. Devising these strategies to target the brain may ultimately become a novel therapeutic modality to treat neurodegenerative diseases.
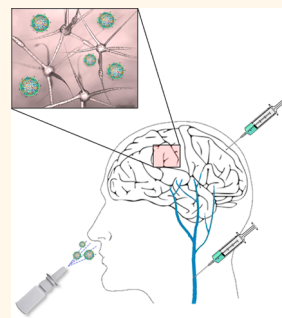
With the worldwide increase of the human lifespan, the frequency and prevalence of neurodegenerative diseases (NDs) is on the rise.[1] "Neurodegenerative diseases" is an umbrella term for a range of conditions, all of which result in a progressive degeneration and destruction of nerve cells. The destruction of nerve cells can produce either ataxia (impairment of movement) or dementia (impairment of memory and cognitive capabilities) and sometimes both. Such symptoms have a debilitating effect on the patient's quality of life and usually end with a lingering death. The most common ND is Alzheimer's disease (AD), which accounts for more then 50% of dementia cases in the U.S.[2] Among the most widespread are also Parkinson's disease (PD), Huntington's disease (HD), and amyotrophic lateral sclerosis (ALS).[3] Despite intensive research, there is currently no effective treatment for NDs, with patients expecting a slow progression toward a debilitated state. Present therapies for NDs focus only on treating the symptoms but do not halt the progression of the disease itself. Finding a treatment that can affect the course of neurodegeneration will likely have a much greater impact on patient survival and quality of life.

The main challenge in the treatment of NDs is to overcome the restrictive mechanism of the blood—brain barrier (BBB) and to deliver therapeutic payloads to the brain. The BBB is the interface between the blood and the brain, preventing the passage of most circulating cells and molecules, thus protecting it from foreign substances and maintaining central nervous system (CNS) homeostasis.[4] As a result, the BBB also prevents an effective systemic delivery of protein therapeutics, such as neurotrophins, enzymes, or antibody domains into the brain. Due to the extreme selectivity at the BBB, often less than 1% of a drug administered through systemic intravenous (IV) injection will reach the CNS, if at all. Therefore, the large systemic doses required to achieve pharmacologically relevant therapeutic levels in the brain could lead to adverse effects in the body.[5] Multiple strategies have been devised in order to overcome the challenges of delivering therapeutic payloads into the CNS.[6]

Strategies for Therapeutic Administration to the CNS. There are two main approaches to deliver therapeutics into the brain: invasive and noninvasive (Figure 1).[5,7]

Invasive drug delivery involves entering the brain using surgical methods such as intracerebroventricular infusion, disruption

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of the BBB, or injecting directly into the target site.[7] An example of an interesting strategy for disruption of the BBB is the use of focused ultrasound that may open the BBB for a designated time while drugs are administered.[8] Invasive approaches can be aggressive (by mechanically breaching the BBB) and aim to maximize the amount of drug that reaches the target site with minimal exposure to the surrounding tissue. This approach is important when treating brain tumors since many chemotherapeutic agents are toxic substances.[9] Invasive approaches limit drugs from entering the systemic circulation, reducing side

effects. However, invasive treatments are not suitable for chronic disorders requiring long-term intervention. These techniques require hospitalization and leave the BBB open, which may cause secondary infections as well as traumatic injury due to mechanical intervention.

Noninvasive drug-delivery approaches, on the other hand, aim to enter or bypass the BBB, utilizing endogenous transport mechanisms.[5] These strategies include pharmacological drug modification approaches, targeting drugs to specific endogenous transporters or carriers, and intranasal drug delivery.[10]

Pharmacological approaches consist of modifying drugs to reduce the exposed polar groups in order for the drug to cross the BBB. The most common chemical approach to deliver drugs across the BBB is by increasing lipophilicity. For instance, creatine is a neuroprotective compound, which cannot pass the BBB due to its polarity. It has been modified into a more hydrophobic derivative, allowing it to cross the BBB and therefore making it suitable for treating acute and chronic NDs.[11] The disadvantages of this approach include loss of desired activity of modified drugs and extrusion of the drug outside

with efflux pump P-glycoprotein (Pgp) due to increased drug lipophilicity.[12,13] Another strategy is to block P-gp to improve the transport of molecules into the brain.[14]

Targeting a drug to specific endogenous transporters represents a novel strategy for CNS drug delivery, and it is achieved by receptor-mediated transport (RMT) or carrier-mediated transport (CMT). Large peptides and proteins can cross the BBB by RMT. Examples of well-known endogenous peptide receptors located at the BBB are the insulin receptor (INSR), transferrin receptor (TFR), and the insulin-like growth factor receptors (IGF1R and IGF2R).[15] Carrier-mediated transport carries small molecules (MW < 600 Da) because it is designed to deliver nutrients, vitamins, and hormones into the CNS. An attractive strategy is to conjugate an endogenous transporter substrate to a drug. In this issue of *ACS Nano*, Song *et al.* describe elegant nanostructures designed to have dual activity: Aβ-binding and permeability of the BBB. These biologically inspired nanostructures, which are based on apolipoprotein E3 reconstituting high-density lipoprotein, were termed ApoE3-rHDL. These nanostructures can capture Aβ in the brain interstitial fluid and enable its degradation in the CNS by glial cells. ApoE3 binds to the LDL-related protein receptor type 1 (LRP1), which mediates endocytosis across the BBB into the brain. ApoE3-rHDL was found to bind with high affinity to both Aβ monomers and oligomers and facilitate their degradation by glial and liver cells. Intravenous (IV) administration of ApoE3-rHDL decreased amyloid deposition, attenuated microgliosis, and rescued memory deficits in an AD animal model.[16] This work may serve as a novel nanomedicine for therapeutic intervention in AD.

Intranasal (IN) delivery of proteins has recently emerged as a noninvasive, safe, and effective method for targeting peptides and proteins to the CNS, bypassing the
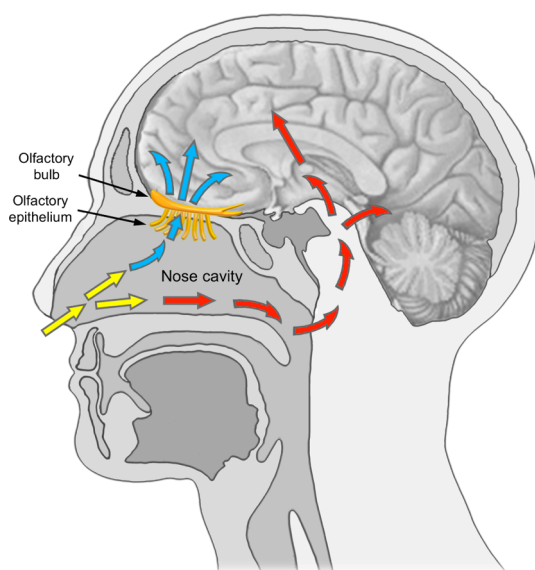
Figure 1. Intranasal delivery pathways. Intranasal applied drugs are rapidly transported into the brain tissue both by the peripheral olfactory route (shown in blue) and by the peripheral trigeminal route (shown in red).

In this issue of *ACS Nano*, Song *et al.* designed elegant nanostructures with dual activity: Aβ-binding and permeability of the blood−brain barrier.

BBB, minimizing systemic exposure, and limiting peripheral side effects.[17] With IN administration, the drug is transported into the CNS through the olfactory epithelium in the nasal cavity. Once the drug passes through the olfactory epithelium, it enters the olfactory bulb and diffuses through the olfactory and trigeminal nerve, effectively distributing the drug throughout the brain.[17]

A wide variety of peptides and proteins that were administered by IN delivery appeared rapidly (in <1 h) in the brain and exhibited pharmacological effects. In some cases, IN administration resulted in higher levels in the brain and blood than those observed after intravenous administration.[18−21] Both vascular endothelial growth factor (VEGF) and transforming growth factor β1 (TGF-β1) were detected 30 min after IN administration in a wide ranged area of the brain, including the olfactory bulb, the striatum, and the thalamus. In both cases, the concentration detected after IN administration was higher than when administered intravenously.[19,22] Vasoactive intestinal peptide (VIP), interferon beta 1b, and insulin-like growth factor-I (IGF-I) have each been administered to rats by the nasal route and rapidly achieved peak brain levels exceeding those seen after direct intravenous administration.[20,21,23−25] In addition, IN nerve growth factor (NGF) was shown to result in CNS concentrations 2−3-fold higher

than those obtained following intravenous administration.[26] Pharmacological activity was also documented for IN administered peptides and proteins, confirming their transport to the CNS. For example, IN delivery of fibroblast growth factor and heparin-binding growth factor stimulates neurogenesis in adult mice.[27] Intranasal administration of NAP peptide, the active form of activity-dependent neurotrophic factor (ADNP), reduced neurodegeneration and amyloid accumulation in mouse models of AD. The NAP peptide was successfully delivered through the IN route, providing neuro-protection against loss of cholinergic functions and memory impairments.[28,29] Insulin distributes widely throughout the mouse brain 1 h following IN administration, with the highest levels detected in the trigeminal nerve and the olfactory bulbs.[30] Levels of insulin in the CNS were significantly higher than levels measured following subcutaneous injection at this time point. IN administration of insulin improved cognitive functioning in clinical trials and increased insulin levels in the CNS in Alzheimer patients.[10,31] Furthermore, insulin improves memory and cognitive function in humans following IN administration,[31−34] without altering plasma insulin or glucose levels.[35]

One of the first characterized proteins delivered to the brain by IN administration was IGF-1. The IGF-1 concentration was found to be significantly higher in rat brains administered *via* IN delivery as opposed to IV administration of the same dose. Delivery was rapid, and distribution was widespread, implying that IGF-1 entered *via* the olfactory and the trigeminal pathways. Moreover, activation of signaling pathways in areas expressing high levels of IGF-1 receptor indicated that the IGF-1 reached its target sites intact.[21]

Antibodies are valuable therapeutic proteins owing to their capability to bind and to neutralize peptides and proteins in a highly

specific manner. However, the high molecular weight of antibodies limits their penetration into the brain following systemic administration. To overcome the size issue and also to avoid the inflammatory properties elicited by the Fc domain, smaller fragments can be engineered. Intranasal administration of antibodies could be an alternative route for immunotherapies. A TNF-α inhibitory single-chain Fv antibody fragment (ESBA105) was administered by IN and by IV delivery into mice, and pharmacokinetic parameters were tested at different brain regions. ESBA105 was detected at significantly higher levels following IN administration, as compared to the levels after IV delivery.[36] These examples validate that the IN administration route may serve as an advantageous approach for the treatment of NDs.

Nanotechnology-Based Drug-Delivery Strategies. Despite the great therapeutic potential that proteins and peptides hold, their application is restricted by low stability in the plasma and poor transfer across biological membranes.[37] Proteins can be stabilized by chemical modifications: by binding proteins to small molecules such as sugars or surfactants or by fusing proteins to other highly soluble and stable proteins such as immunoglobulins or albumin. Although these methods enhance protein stability, they can hinder protein activity.[38] Another method for protecting and transporting proteins until they reach their site of action is to encapsulate them within nanoparticles (NPs). Nanoparticles can be constructed of a variety of natural or synthetic materials and range in sizes between 10 and 1000 nm.[39,40] Nanoparticles have been researched extensively as delivery platforms for chemotherapy, anti-inflammatory, and other small molecular drugs. They also have been shown to be an effective delivery platform for large macromolecules such as proteins and polynucleotides.[41] Nanoparticles have been successfully modified with
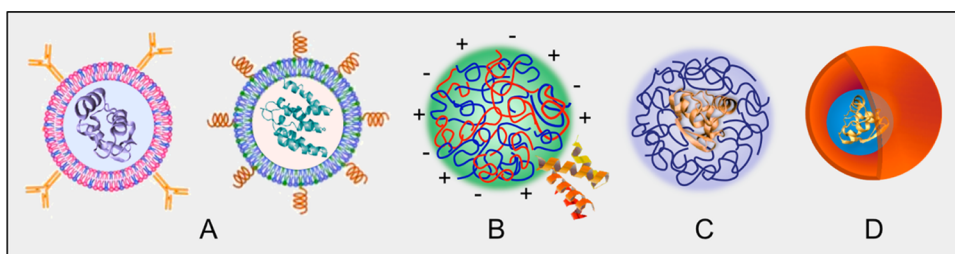
Figure 2. Graphical representation of nanoparticles utilized for protein delivery. (A) Liposomes either conjugated to antibodies to enable targeted delivery or conjugated to polymers to enhance blood−brain barrier penetration. (B) Biodegradable polymer nanospheres. (C) Biodegradable polymer nanocapsules. (D) Solid lipid particles.

targeting moieties that enable them to deliver drugs to specific tissues or to pass through mucosal barriers in the body such as the intestinal lining.[42]

Numerous NP formulations (Figure 2) have been produced to perform different tasks from drug delivery to contrast imaging. Formulations include lipid-based NPs (micelles, liposomes, solid lipid particulate systems, lipospheres, microspheres, and hybrid lipid−polymer systems), polymeric NPs (PPs), niosomes, gold NPs, and carbon nanostructures. There are also a variety of production methods to manufacture NPs. Even NPs constructed from the same building blocks can have extensive modification of their structure and physiological properties depending on the manufacturing method.[43] Regardless of their composition or assembly method, in order for a NP to be an effective carrier, it must display several basic characteristics: high stability to protect its cargo, biodegradability in order to release its therapeutic payload at the site of action, and biocompatibility by showing low to no toxicity and minimal immunogenicity (if any). Additionally, in order for NPs to be used as effective pharmaceutical products, the control, manufacturing, and chemistry (CMC) processes need to be worked up for large-scale production and must also be cost-effective.[42]

Most NPs were designed with the intent to deliver small molecules. Protein encapsulation is more complex, and therefore, several key factors need to be taken into account, such as the relatively large size of the protein, the hydrophilic nature of most proteins, and the fact that most proteins, and especially enzymes, are sensitive to environmental factors. Great care should be taken during the encapsulation of the protein within NPs to prevent extreme physical conditions such as high temperature, pH, or ionic strength, all of which can cause protein deterioration and aggregation. For example, lipid-based NPs (LNPs) are often constructed with the use of nonaqueous solvents and detergents and then are downsized to nanometric dimensions using physical procedures such as sonication and extrusion. All of these processes can damage encapsulated proteins, reducing their biological activity and triggering immunogenic responses.[37]

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Several NP formulations have been investigated for protein encapsulation, specifically, liposomes, PPs, and solid lipid nanoparticles (SLNs). Liposomes are the best recognized and most investigated delivery systems for proteins and small molecules. They have been extensively and successfully used to deliver small molecule drugs displaying controlled release and site-specific drug delivery. By changing the composition of the phospholipids or the method of liposome preparation, it is possible to change the physicochemical properties of the liposomes such as charge, structure, and dimensions,[44] thus enabling a wide range of possibilities to suit encapsulation of different proteins. Liposomes have successfully encapsulated a wide variety of proteins including superoxide dismutase,[45] acetylcholinesterase,[46] and myoglobin.[47] Liposomes are able to facilitate tissue and cellular penetration by several mechanisms, one of which is endocytosis, due to their lipophilic features.[48] In addition, liposomes have been used successfully for BBB transport delivering anticancer and anti-inflammatory drugs (paclitaxel, curcumin, and doxorubicin)[49] into the brain by IV or intracerebral delivery.[50]

Polymeric particles can be produced with a wide variety of materials ranging from synthetic biodegradable polymers such as polylactide (PLA) and poly(lactide-co-glycolide) (PLGA),[51,52] to natural polymers such as polyesters (poly(lactic acid), amino acids) and polysaccharides (alginate, hyaluronic acid, dextrans, and chitosan),[51,53] with or without stabilizers, such as poly(ethylene glycol) (PEG). Even proteins themselves have been used to create protein NPs (gelatin, albumin).[54] Polymeric particles can be generally classified into two different types: nanospheres and nanocapsules. In nanospheres, drugs are either adsorbed or entrapped within the polymeric

matrix, whereas in nanocapsules, drugs are confined to the inner liquid core while the polymeric membrane covers the external surface of the particle. While logically it would seem that nanocapsules should be better protein carriers due to their large hydrophilic compartment, both nanocapsules and nanospheres have successfully entrapped proteins.[55]

Solid lipid nanoparticles are relatively new to the field of protein encapsulation. Solid lipid nanoparticle systems were devised with the intent to encapsulate hydrophobic drugs for injection or infusion. As a delivery system, SLNs have several advantages over other carrier systems due to their high physical stability, controlled release, and low toxicity,[56] although because of their hydrophobic nature, they were thought not to be suited for protein encapsulation. The application of the double-emulsion (w/o/w) technique has enabled encapsulation of hydrophilic molecules, allowing the encapsulation of several therapeutic proteins such as lysozyme, BSA, HSA, thymocartin, and somatostatin.[38]

In order to enable NPs to cross the BBB, they can be coated or bound to a variety of molecules that enable passive or active BBB transport. Passive BBB transport can be achieved by binding cationic moieties or surfactant molecules to NPs; this enables adsorptive-mediated transcytosis into the cells that compose the BBB and thus enables the NPs to enter the brain *via* simple diffusion. Active BBB transport can be facilitated by binding ligands that promote direct interaction with BBB transport systems. They can be natural ligands such as insulin or opioid peptides, or they can mimic the effect of natural ligands such as antibodies that target specific receptors. Another method is to coat the NPs with molecules that enable stealth capabilities and prevent absorption and clearance from the blood. The most common molecule for such coating is PEG. Poly-(ethylene glycol)-covered NPs are widely used in drug-delivery

systems and liposome-based delivery systems. *In vivo* experiments revealed that NPs coated with PEG successfully penetrated the BBB with superior results to other chemical modifications.[57] Other strategies include the use of the rabies peptide (RVG) to cross the BBB.[58] Rabies peptides were conjugated to siRNA-encapsulating exosomes. The targeted exosomes were able to target and specifically silence specific genes in neurons, microglia, and oligodendrocytes, demonstrating the high potential for peptide-mediated delivery.[59]

Combinational Therapy of Nanotechnology and IN Administration. Nerve growth factor is another important neurotrophic factor, efficient for the treatment of NDs such as AD and PD. The delivery of NGF to the brain has been shown by several approaches, including direct injection and IN administration.[59] Recombinant human NGF (MW = 26.5 kDa) was found to reach wide ranges of the brain, including the olfactory bulb, cortex, and hippocampus, and at a higher concentration following IN administration as compared to the same dose by IV administration.[19] Moreover, IN delivery of NGF in an AD mouse model showed decreased Aβ accumulation and rescue of recognition memory deficits.[23,60] However, none of the above approaches have been proven to be effective for long-term application. Nerve growth factor loaded in polysorbate 80-coated NPs resulted in significant reduction of basic PD symptoms, such as reduction in rigidity and improvement of locomotor activity. The effects were observed even 1 and 3 weeks after administration.[61] These results can be related to the ability of the polysorbate 80-coated NPs to enhance transport into the brain. This coating leads to the adsorption of apolipoproteins such as A-1, B, and E onto the surface of the NPs after IV administration.[62−64] These proteins interact with their specific receptors on endothelial cells and promote endocytosis of the NPs by these cells.

Nerve growth factor loaded in polysorbate 80-coated NPs reversed scopolamine-induced amnesia and improved recognition and memory in an amnesic mouse model.[65] From these results, it can be concluded that NPs coated with polysorbate 80 are effective carriers for transporting NGF to the CNS following IV administration and may serve as a long-term treatment for NDs. In 2005, Xie *et al.* successfully transported NGF encapsulated into receptor-targeted liposomes across the BBB following IV administration in rats. RMP-7, a ligand to the B2 receptor on brain microvascular endothelial cells, was conjugated to DSPE-PEG and incorporated into the liposome surface in order to target it to the brain by crossing the BBB. The highest concentration of NGF was detected 30 min after IV administration. Nerve growth factor was distributed in the striatum, hippocampus, and cortex, and relatively low levels were found in the olfactory bulb, cerebellum, and brain stem; however, other organs also absorbed relatively high amounts of NGF, including liver, spleen, lung, and kidney.[66] Substituting IV with IN administration of NGF encapsulated within carriers should be more advantageous due to rapid local delivery, enabling slow release and eliminating systemic effects.

Chick ovalbumin (MW = 45 kDa) was chosen as a model protein for IN protein delivery. It was successfully observed in the substantia nigra and striatum after IN administration. Higher levels of ovalbumin were detected when entrapped in cationic liposomes. This was the first documented study of IN administration of a protein entrapped within nanocarriers. Evidently, cationic liposomes enhance brain transport and extend brain residence time compared with unencapsulated protein. Due to the involvement of the substantia nigra and striatum in the pathogenesis of PD, the IN route may hold great promise for the treatment of PD.[67] This work opens the possibility that combinational

therapy of both IN administration and nanocarrier protein encapsulation provides an effective strategy to deliver large proteins to the brain for the treatment of NDs.

FUTURE OUTLOOK

NDs are becoming one of the major health problems of our times. Not only is the occurrence of NDs on the rise due to global aging of the population, but there are no current medical treatments that can prevent the initiation of NDs or delay their progression. Today, there is an immediate need for development of novel therapeutics for NDs—therapeutics that will affect disease outcome rather than just treating the symptoms.

Nanomedicine is at the forefront of the development of such therapeutics and especially the development of procedures to transport neuroactive proteins and peptides into the brain. Protein-based therapeutics such as enzyme replacement therapy and antibody-mediated therapy could hold the key for the treatment of NDs by restoring functionality and activity of neurons and glia cells. Tremendous efforts over the last decades have resulted in numerous strategies to facilitate protein delivery to the brain, but no single method is without its limitations or drawbacks. In order to devise better delivery methods, the nanomedicine community needs to learn more about the physiology and pathology of the brain. The challenges for effective protein transport are far beyond the mere understanding of the physical and chemical aspects of nanocarrier construction. We also need to understand the complex milieu of the brain, including the function of glial cells, their interactions with neurons, how to manipulate and to restore function of motor and sensory neurons, and how to bypass or to transport proteins into the brain. Great attention should be given to the combination of alternative routes of administration, primarily IN delivery and nanocarriers that exploit physiological processes such as the BBB receptor-mediated transport. The discovery of new potential therapeutic targets should be hypothesized, explored experimentally, validated in preclinical models, and eventually tested clinically with the prospect that, one day, it will provide an effective treatment for NDs.

Conflict of Interest: The authors declare the following competing financial interest(s): D.P. has financial interest in Quiet Therapeutics.

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