Expert Opinion

Challenges and opportunities in CNS delivery of therapeutics for neurodegenerative diseases

Thomas M Barchet & Mansoor M Amiji†

Northeastern University, School of Pharmacy, Department of Pharmaceutical Sciences, Boston, MA 02115, USA

With an increase in lifespan and changing population demographics, the incidence of central nervous system (CNS) diseases is expected to increase significantly in the 21st century. The most challenging of the CNS diseases are neurodegenerative diseases, characterized by age-related gradual decline in neurological function, often accompanied by neuronal death. Alzheimer's disease, Parkinson's disease and Huntington's disease are some examples of neurodegenerative diseases and have been well described in terms of disease mechanisms and pathology. However, successful treatment strategies for neurodegenerative diseases have so far been limited. Delivery of drugs into the CNS is one of the most challenging problems faced in the treatment of neurodegeneration. In this review, we describe the difficulties with CNS therapy, especially with the use of biological macromolecules, such as proteins and nucleic acid constructs. CNS therapeutics also represents a huge opportunity and examples of strategies that can enhance therapeutic delivery for the treatment of neurodegenerative diseases are emphasized. It is anticipated that with an increase in biological understanding of neurodegenerative diseases, there will be even more therapeutic opportunities. As such, these delivery strategies have a very important role to play in the future in the translation of CNS therapeutics from bench to bedside.

Keywords: Central nervous system, drug delivery systems, intranasal delivery, nanotechnology., neurodegenerative diseases, systemic delivery

Expert Opin. Drug Deliv. (2009) 6(3):211-225

1. Introduction

It is estimated that as many as 1.5 billion people worldwide are suffering at any given time from some type of central nervous system (CNS) disorder [1]. The situation is expected to worsen significantly in the 21st century when more individuals are expected to live past 70 years of age, the point at which approximately 50% of the population starts to develop symptoms of Alzheimer's disease [2]. CNS disorders currently represent 11% of the global burden of disease, which is expected to rise to 14% in 2020, driven to a considerable extent by the aging of population [3]. Neurodegeneration is a common theme of many CNS diseases, such as Alzheimer's disease, Amyotrophic Lateral Sclerosis (ALS or Lou Gehrig's disease), Huntington's disease, Parkinson's disease, head trauma, epilepsy and stroke. These disorders are devastating and expensive, with annual costs currently exceeding several hundred billion dollars in the US alone, and current treatments are inadequate. Adding to the urgency of the problem is the fact that the incidence of these age-related disorders is increasing rapidly as population demographics change. In the US, for instance, 1,600 out of every 100,000 people develop Alzheimer's disease, 110 out of every 100,000 people develop Parkinson's

10.1517/17425240902758188 © 2009 Informa UK Ltd ISSN 1742-5247

disease, and 5 – 7 out of every 100,000 people develop Huntington's disease. **Table 1** below shows a summary of various neurological diseases and the representative statistical data in the US.

The inadequacy of current therapeutic strategies for neurodegenerative diseases is largely based on the fact that they are meant to alleviate the symptoms, but they do not have any disease-modifying effects. Additionally, the doses and number of drugs required for chronic lifelong treatment lead to lifestyle altering and often debilitating side effects. The newer generation of biological therapeutics, based on peptides, proteins and nucleic acid constructs, can have a profound influence on neurodegenerative diseases, as these newer therapeutics have the potential for disease modifying effects. These new treatments are both potent and specific, as they act at the level of the disease-causing molecules. Many types of protein-based therapeutics provide a unique strategy for arresting disease progression and even restoring the function of damaged neurons. More than 30 different neurotrophins, including nerve growth factor (NGF), glial cell-derived neurotrophic factor (GDNF), brain-derived nerve growth factor (BDNF) and basic fibroblast growth factor (bFGF), have been identified [4,5]. Additionally, gene therapy can potentially protect against a number of neurodegenerative diseases by delivering genes encoding for neurotrophins, enzymes, anti-oxidants, anti-inflammatory and anti-apoptotic molecules [1,6-10]. Small interfering RNA (siRNA) that can silence specific genes, such as β-amyloid and α-synuclein for Alzheimer's and Parkinson's diseases, respectively, are also of significant interest in neurodegenerative diseases.

2. Neurodegenerative diseases

Although neurodegenerative diseases comprise a large number of different pathologies that ultimately lead to loss of neuronal activity in the CNS, emphasis in this review is placed on Alzheimer's disease, Parkinson's disease and Huntington's disease, based on their prevalence. Additionally, examples of therapeutic strategies focused on biological macromolecules with disease modifying effects are highlighted. **Table 2** shows several important examples of biological macromolecules that are investigated in neurodegenerative diseases.

2.1 Alzheimer's disease

Alzheimer's disease (AD) is a neurodegenerative disease characterized by the formation of plaques and neurofibrillary tangles composed of β-amyloid and tau proteins, respectively [11,12]. AD is the most common form of dementia, and the greatest risk factor for AD is age. It is estimated that 10% of people over the age of 65 are affected by AD [13] and by 2050, the number of people with AD in the US will increase threefold [14]. All current therapies are used to treat the symptoms of AD. The acetylcholinesterase inhibitors donepezil, galantamine, rivastigmine and tacrine and the NMDA receptor antagonist memantine are the five medications currently approved by the Food and Drug Administration (FDA) for use for the treatment of AD [15]. Anti-psychotics have been prescribed as off-label treatments for AD; however, their efficacy has been refuted [16]. Curcumin, a molecule from the Indian curry spice, is under investigation for AD treatment, as it has been shown to have anti-inflammatory properties and may decrease the amyloid plaque burden in an animal model of AD [17]. The omega-3 fatty acids, such as docosahexaenoic acid and α-linolenic acid, have also been linked to a decrease in the levels of amyloid in an AD model [18].

There are several drugs that are currently in development to treat AD. While some of these drugs are symptom-management therapeutics, there are several candidates that directly alter the disease progression. Newer biological molecules have also been developed as potential treatments for AD. The recombinant DNA drug etanercept (Enbrel®: Immunex Corporation, Thousand Oaks, CA) is a potent inhibitor of the pro-inflammatory cytokine, tumor necrosis factor alpha (TNFα). It is indicated for use in diseases with excess inflammation, such as rheumatoid arthritis and auto-immune disease. It has been found that peri-spinal administration of etanercept can improve the progression of AD by interfering with the high TNFα levels that are associated with AD [19]. Nerve growth factor (NGF) gene therapy has also been used in clinical trials for the treatment of AD to prevent the degeneration of cholinergic neurons [20]. Of the patients that underwent successful NGF treatment, there was an improvement in cognitive decline [21]. However, there was significant morbidity and mortality associated with the intracranial delivery strategy using the patients' NGF encoding fibroblasts. The humanized monoclonal antibody, bapineuzumab or AAB-001, is being jointly developed by Elan and Wyeth as a treatment for Alzheimer's disease [22]. Bapineuzumab is thought to act by binding to β-amyloid plaque and clearing it from the CNS. Other anti-TNF strategies, such as administration of TNF-silencing siRNA or low molecular TNF inhibitors can also have profound impact in reducing inflammatory effects in AD.

While there is a great amount of work that has been done in the treatment of AD, there have not been any treatments that have stopped the progression of the disease. Currently, a variety of treatment options may need to be combined in order to achieve successful therapy. Overall, successful therapy may require simultaneous treatment of symptoms, as well as direct modification of the disease.

2.2 Parkinson's disease

Parkinson's disease (PD) is characterized by the loss of dopamine neurons in the substantia nigra [23]. This has been associated with accumulation of ubiquitinated α-synuclein in the dopaminergic neurons [11]. Estimates state that 1% of people over age 60 are affected by PD, the leading cause of

Table 1. Incidence of selected neurodegenerative diseases in the US.

Disease	Incidence (per 100,000 population)	Ref.
Alzheimer's disease	1,600	[14]
Parkinson's disease	110	[103]
Multiple Sclerosis	95	[104]
Huntington's disease	5 – 7	[27]
Amyotrophic Lateral Sclerosis	1.5	[105]

Table 2. Selected examples of biological therapies for neurodegenerative diseases.

Treatment option	Disease	Ref.
Monoclonal antibodies		
Bapineuzumab	Alzheimer's disease	[15]
LY2062430	Alzheimer's disease	[15]
RN1219	Alzheimer's disease	[15]
Growth factors		
Glial cell-derived nerve growth factor	Parkinson's disease	[106,107]
Brain derived growth factor	Parkinson's disease	[106]
Ciliary neurotrophic factor	Huntington's disease	[107]
Nerve growth factor	Alzheimer's disease and Huntington's disease	[107,108]
RNAi		
shRNA	Huntington's disease	[30]
Immunization		
AN-1792	Alzheimer's disease	[15]
ACC-001	Alzheimer's disease	[15]

the movement disorder called Parkinsonism [13]. Parkinsonism consists of tremors, bradykinesia and, in extreme cases, akinesea [23]. As there is currently no cure for Parkinson's disease, and there are no known agents to slow the progression of PD; the current treatments are used to decrease the symptoms of PD. There are a variety of treatments available, including the dopamine precursor levodopa, catechol-*O*-methyl transferase (COMT) inhibitors, dopamine agonists, anti-cholinergics, amantadine and monoamine oxidase (MAO) inhibitors [24].

Various types of growth factors (e.g., glial cell-derived growth factor, brain derived growth factor, etc) have shown to be very efficacious in neuroprotection for PD [25]. Additionally, a newer generation of nucleic acid constructs can silence α-synuclein. It has been suggested that RNAi could be applied to the treatment of PD, and it has been shown that in animal models of PD, successful treatment can be achieved [26]. These novel therapeutics require advanced delivery methods, as they will need to enter the CNS to have their effects. The use of the intranasal route or nanocarrier devices discussed below not only can protect these drugs from degradation and clearance; they could also increase the penetration of the large biological molecules into the CNS.

2.3 Huntington's disease

Huntington's disease (HD) is characterized by the aggregation of a mutant Huntingtin protein in the neurons, leading to improper neuronal function and eventually neuronal death [27]. This leads to abnormal movements in the patient, referred to as chorea [28]. Huntington's disease is caused by an autosomal dominant mutation, and the disease is currently incurable. Although the genetic and cellular components of HD have become more understood, the current treatments that are available treat the symptoms, not the disease itself. Accompanying the chorea experienced by HD patients, there are often neurological problems, most notably depression and psychosis [29]. A variety of neuroleptics are used to treat the chorea associated with HD [29]. Some HD patients

experience epilepsy, and they are treated with typical anticonvulsants, such as valproic acid [29]. Parkinsonism can be observed in HD, and levodopa is a common treatment for these symptoms [29]. Although there is no current treatment available to target the mutant Huntingtin protein, there are currently several disease modifying treatments in development. The use of recombinant adeno-associated virus to deliver RNAi therapeutics has been shown to decrease pathology in a disease model of HD [30]. Neural implant of ciliary neurotrophic factor secreting cells has been associated with an improvement in HD. Overall, new therapeutics must be designed to protect the degrading nervous system in HD. Neuroprotective and disease modifying therapies are promising treatment avenues for HD. One possibility is that through the use of novel delivery strategies with siRNA, it may be possible to deliver therapeutics to the CNS to knock out the mutant Huntingtin protein and prevent the progression of HD.

3. Barriers to CNS delivery

Drug delivery to the brain is one of the most critical obstacles to CNS therapy. It is estimated that 98% of small molecule drugs and 100% of proteins and nucleic acid therapies are excluded from the CNS due to delivery barriers [31]. This is due to the blood–brain barrier (BBB), the blood–cerebrospinal fluid (CSF) barrier, and dilution effects due to the systemic distribution of drug. **Figure 1** below shows a schematic of the BBB and illustrates both the physical barrier in the form of tight cellular junctions and astrocyte 'feet', as well as the biochemical barrier in the form of efflux transporters and metabolizing enzymes expressed on the luminal surface of brain capillaries.

3.1 The blood–brain barrier

The BBB is the term used to describe how molecules distribute to most tissues of the body, but they are unable to penetrate into the brain. The phenomenon was first described by Paul Ehrlich. He observed that when water soluble dyes were injected into the circulatory system, the dye stained all tissues except for the brain and spinal cord [32,33]. Edwin Goldmann followed up on the study by injecting trypan blue directly into the cerebral spinal fluid (CSF). In this study, the dye stained the brain and CNS, but it failed to penetrate into the rest of the tissues [33]. These experiments led to the theory that there is a mechanical barrier that separates the circulatory system from the central nervous system. This mechanical barrier has been attributed to the formation of tight junctions between the endothelial cells of the brain microvasculature [34]. These tight junctions consist of claudin and occludin proteins which interact to create a virtually impenetrable barrier between the blood and the brain [35]. From this initial discovery, it has been found that the BBB is not only a mechanical barrier, but it is also an active chemical and metabolic barrier. There are active mechanisms by which the BBB excludes compounds from the brain such as P-glycoprotein (P-gp) and multidrug resistance-associated proteins [36]. The cells of the BBB also exhibit high levels of metabolizing enzymes such as those of the cytochrome P450 family [37,38]. These barriers all contribute to the function of the BBB. Due to these properties, it has been postulated that BBB penetration requires a drug to have none of the following: a molecular weight greater than 500 Daltons, more than 10 hydrogen bond donors/acceptors, affinity for the BBB enzyme system, affinity for the BBB efflux system, or high binding to plasma proteins [39]. The BBB is the most direct barrier between the CNS and systemic circulation. Overcoming the BBB should, therefore, be of greatest interest in the development of CNS drug delivery systems.

3.2 The blood–CSF barrier

The blood–CSF barrier is the term used to describe the barrier between the systemic circulation and the cerebrospinal fluid. Like the BBB, it excludes chemicals from the CNS. It acts as a selective filter allowing some molecules through to form the cerebrospinal fluid. Tight junctions between the epithelial cells of the choroid plexus form the mechanical barrier. It is worthwhile to note that there are not tight junctions in the capillaries that supply the choroid plexus, but rather there are fenestrae between the capillaries that supply this region. Drug and other molecules can freely diffuse from the capillary through these fenestrae, but they are prevented from entering the CSF due to the epithelial tight junctions. Another noteworthy characteristic of the blood–CSF barrier is its surface area relative to the BBB. The blood–CSF barrier has 1,000 times less surface area than the BBB [40], therefore, in terms of drug delivery, the blood–CSF barrier is a less significant obstacle to delivery.

3.3 Systemic distribution and clearance

One frequently overlooked barrier to CNS delivery is the dilution effects and metabolism/degradation due to systemic distribution and clearance of drugs in peripheral tissues. As the approach of increasing lipophilicity of drug molecules has been used to increase CNS permeability, these lipophilic compounds more readily penetrate into all other tissues in the body when administered into the systemic blood circulation. With the increase in systemic distribution, a larger dose must be given to achieve the required therapeutic levels in the brain. This leads to non-specific systemic effects and increased systemic toxicity. As previously stated, high plasma protein binding affinity will make drug delivery to the CNS difficult, as there is little free drug to diffuse into the CNS. Although this is frequently considered a minor barrier to CNS delivery, it is one area that could benefit tremendously by using novel drug delivery systems. By encapsulating the therapeutic agent in a carrier, premature release in the systemic circulation and the overall pharmacokinetic profile are altered.

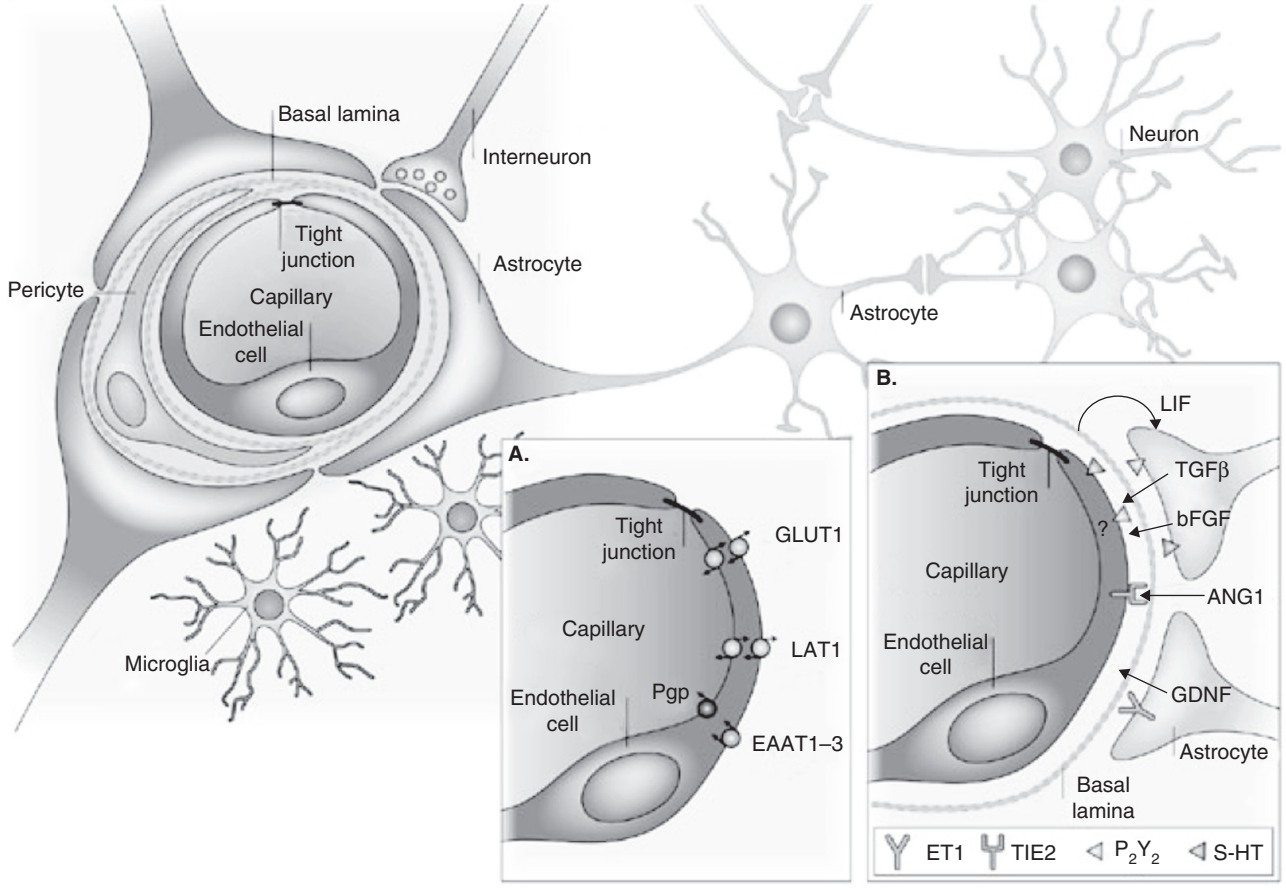

Figure 1. Schematic model of the blood–brain barrier (BBB) formed by capillary endothelial cells, surrounded by basal lamina and astrocytic perivascular end-feet. In addition to being a physical barrier in the form of tight endothelial cell junctions (a), the BBB is also a formidable biochemical barrier, with expression of various efflux transporters such as P-glycoprotein (Pgp) (b).

Reprinted by permission from Macmillan Publishers Ltd: Nature Reviews Neuroscience 2006;7:41-53

4. Strategies to enhance CNS delivery

There are a variety of approaches that have been examined in overcoming the barriers to CNS delivery. Each of the strategies discussed below has advantages and disadvantages and these are highlighted with relevant examples from the literature. The majority of the discussion here is focused on strategies that utilize active therapeutics for: i) invasive versus non-invasive delivery; and ii) systemic versus local administration.

4.1 Intracranial delivery

Intracranial delivery is an invasive method by which the CNS barriers can be overcome through direct administration requiring a surgical procedure. This involves the use of intracerebral infusions or implants to administer the drug directly into the brain parenchyma. One drawback is that the delivery is localized to the administration site [41,42]. It has been shown that with intracranial delivery, the diffusion of the drug to other areas of the brain is limited. The disease being treated is also critical to the potential success of intracranial delivery. Intracranial can be advantageous for certain targets, such as local tumor delivery via a drug releasing implant or through direct treatment of the failing neurons in the substantia nigra in PD; however, this method does not deliver to a broad range of neural tissue as would be desired in the treatment of AD or HD. Another major drawback is the method of administration. Intracranial delivery requires invasive surgery. There is not only increased risk involved with these surgeries, but there can also be greater variability in the administration of drug.

Although intracranial delivery has many obstacles, it has been shown that administering neurotrophic factors directly to the brain can be accomplished with positive outcomes. A neural implant of GDNF producing cells has been shown to correct movement disorders associated with neurodegeneration in aged rats [43]. Direct striatal injections of GDNF encoded by lentivirus have also been shown to

prevent neurodegeneration in both aged and NPTP models of PD in rhesus monkeys [25]. This is an important finding in the treatment of PD, as it shows that the local administration resulted in successful treatment, without the accompanying side effects observed after widespread administration of GDNF to the entire brain.

Although there are a number of preclinical and clinical studies with direct intracranial administration of therapeutic agents, some the most important evidence comes from Amgen's trial of GDNF in PD patients. Amgen sponsored the first multicenter, double-blind, placebo-controlled trial involving intrastriatal administration of recombinant methionyl human GDNF (r-metHuGDNF; Liatermin®: Amgen Inc., Thousand Oaks, CA, USA). Thirty-four patients were randomized to receive either GDNF (15 µg/striatum/day), or placebo bilaterally. Unexpectedly, GDNF treatment was found not to be significantly different from placebo as assessed by the motor scores, even though a significant difference of 32.5% in [^{18}F]-dopamine influx constant was observed in the GDNF treatment group [44]. However, nine patients experienced 'device-related serious adverse events', two patients required catheter repositioning, one patient required catheter removal and one patient suffered a hemorrhagic stroke. These findings, together with the results of a 6-month toxicology study in monkeys, which showed Purkinje cell loss following intrastriatal administration of GDNF, prompted Amgen to abruptly discontinue its GDNF clinical trial in February 2005 [44,45]. What is absolutely clear from this clinical trial is that the intrastriatal route of administration in Parkinson's patients resulted in several unacceptable adverse effects and an inconsistent clinical response.

Intracranial implants have also been used in clinical trials. Genetically engineered cells encapsulated in a polymer matrix have been implanted into patients with HD. These cells have been designed to release human ciliary neurotrophic factor (CNTF). It was shown that this treatment was safe; however, there were no significant clinical benefits to the treatment [46]. It was suggested that this might have been due to variable release from the implant. Clinical trials have begun on intracranial injection of NGF for the treatment of AD. In the Phase I clinical trials, it has been shown that sterotaxic injection of nerve growth factor into the brain causes a decrease in the rate of cognitive decline in patients with mild AD, with the potentially lethal side effects described earlier [21].

4.2 Transient BBB disruption

Another method of increasing drug penetration into the CNS is by temporary disruption of the BBB. One approach for BBB disruption is through the administration of hyperosmolar agents, such as 20% (w/v) mannitol, directly into the carotid artery. As the hyperosmolar solution enters the vasculature, it is thought that the endothelial cells shrink, causing transient openings of the BBB [47]. This is an effective method; however, it introduces plasma proteins and other potentially neurotoxic molecules to the CNS [48]. Arterial administration of hyperosmolar solutions also leads to significant pain and has been linked to patient mortality.

The use of organic solvents and certain surfactants in drug delivery systems also causes transient BBB opening. It has been shown that co-administration of drugs with ethanol or dimethylsulfoxide causes pharmacological activity in the CNS, indicating that the BBB has been affected by the use of these solvents [49,50]. Non-ionic surfactants have also been used to increase BBB penetration. Polysorbate 80 (Tween® 80: Croda International Plc, East Yorkshire, USA) has been shown to alter the BBB *in vitro* [51]. It has also been found that this interaction is specific to the efflux transporters, and not due to mechanical BBB disruption [52].

Immune adjuvants have also been used to disrupt the BBB. Freund's adjuvant was used in the vaccine for Alzheimer's disease [53]. The mechanism of Freund's adjuvant is twofold. As it is an immune system adjuvant, it recruits an immune response at the injection site, increasing the likelihood of an immune response to the antigen, $A\beta_{42}$. It also acts to cause inflammation, causing the BBB to open, increasing penetration of antibodies to the CNS.

Ultrasound has also been successfully employed to open the BBB. It was shown that local application of ultrasonic waves caused dyes to penetrate into the CNS [54]. In this example, it was shown how local ultrasound administration caused transient BBB opening in an Alzheimer's disease model. This would allow for direct drug administration to the β-amyloid plaques in AD. The duration of BBB opening must be considered when using this method, as well as how the ultrasound affects healthy neural tissue. In order for this method to work, it would need to be developed such that it briefly disrupts the BBB for drug administration, causes no neural damage, and does not leave the BBB open long enough for toxins to accumulate in the brain.

The risks associated with BBB disruption must be carefully considered before it is proposed for use in the treatment of chronic neurodegenerative diseases. It is not guaranteed to achieve the widespread effects that are desired for complete brain delivery. Another critical consideration is the drug administration schedule for neurodegenerative disease treatment. The long-term treatment does not lend itself to BBB disruption, as each time the BBB is disrupted; there is the risk for toxic compounds and pathogens to enter into the CNS. Although it may be possible to increase the concentration of drug that reaches the brain, it also allows a host of other compounds to enter the brain. This has been seen in the failed β-amyloid immunization that was linked to encephalitis [55]. In summary, the methods of transient BBB disruption would need to be dramatically improved before they have a future in human trials.

4.3 Chemical structure modification and co-administration of P-glycoprotein inhibitors

Due to the complications of intracranial delivery and BBB disruption, chemical modifications in drug design have been

applied to increase BBB penetration. It was found that lipophilic compounds more readily penetrate the lipid bilayer of endothelial cells, allowing for better penetration into the CNS. Although it is true that lipophilic compounds are more likely to enter the CNS, high lipophilicity does not guarantee that the compound will have CNS penetration. In addition, lipophilicity may actually enhance peripheral drug distribution and limit availability of the drug in the brain. There are several metabolic and kinetic barriers that exclude lipophilic compounds. For example, levodopa (XLogP = -1.8) is less lipophilic than dopamine (XLogP = 0.9) [56]. However, levodopa readily penetrates the BBB whereas dopamine does not. This is due to the presence of the enzyme monoamine oxidase-B at the BBB [57]. Although dopamine enters the endothelial cells of the BBB, monoamine oxidase-B breaks down dopamine into inactive metabolites [58].

There are numerous transporters that act to supply nutrients and remove compounds from the CNS. One approach to increase BBB penetration is through the chemical modification of the compound to increase its affinity for influx or decrease its affinity for efflux transport. This has been successfully deployed with regards to levodopa. L-DOPA penetration into the CNS is enhanced by the 4F2hc/LAT1 complex [59]. Glucose transporters have also been utilized to enhance BBB penetration. By using an active compound and modifying it to make it chemically resemble a naturally transported molecule, increased brain penetration occurs [60].

One of the most exciting recent advancements in CNS therapeutics involves siRNA delivery. It has been shown that by associating siRNA to a brain penetrating peptide, the peptide carries the siRNA into the CNS [61]. This is an area of great interest to neurodegeneration as AD, PD and HD are all associated with protein malfunction. By using gene silencing in these patients, it may be possible to treat some of these diseases by downregulating the production of the offending protein. This is similar to the 'molecular Trojan horse' approach of delivering molecules across the BBB. It has been shown that by conjugating BDNF to an antibody for the BBB transferrin receptor, there was enhanced penetration of BDNF across the BBB and increased bioavailability in the CNS [62].

Another way of enhancing CNS delivery is through the blocking of efflux pumps. A wide variety of drugs are substrates for P-glycoprotein efflux pumps [63]. These pumps are present at a high concentration in the endothelial cells of the BBB [63]. Drugs can either be modified so that they are not a substrate for the efflux pump, or a drug that inhibits efflux pumps may be given. By administering quinidine with the peripherally active opiate, loperamide, CNS effects of loperamide were observed [64]. This approach provides preliminary evidence that by blocking efflux transporters, one can achieve greater CNS effects.

By coupling several of these strategies together, a drug can be designed to have enhanced BBB penetration through small molecule carriers, as well as enhanced brain retention. The 'lock-in' mechanism proposed by Bodor uses drug modifications to achieve these goals. By preparing a molecule that can penetrate the BBB, and then be metabolized into an non-penetrating molecule, the drug can be retained in CNS tissue [65,66].

4.4 Intranasal delivery

Intranasal delivery is an area of great interest for CNS delivery. This interest is partly due to the evidence suggesting nerves that project into the nasal cavity penetrate through the BBB, as shown in **Figure 2**. If that is the case, it would allow the drug to pass directly from the olfactory region of the nasal cavity into the brain tissue, bypassing the BBB [67]. Anatomically, the olfactory epithelium is situated just below the cribiform plate of ethmoid bone separating the nasal cavity from the cranial cavity [68]. The olfactory sensory cells are bipolar neurons with single dendritic processes extending from the cell body to the apical surface of the mucosa, where they terminate in diminutive non-motile cilia. At the basal end, the neurons terminate into fine non-myelinated axons integrating with other axons to form bundles that penetrate into the cranial cavity through small holes of the cribiform plate. The exact mechanisms by which intranasally delivered drugs traverse the olfactory epithelium to reach the brain and CSF are not completely understood. However, it is generally accepted that transport of solutes from the olfactory epithelium to the brain can occur via three distinct pathways [69-73]. One is the systemic pathway by which small, lipophilic drugs can be absorbed into capillaries of the olfactory epithelium and subsequently reach the brain by crossing the BBB. The other two pathways are slower, but are more likely to be involved in the brain uptake of large molecular weight solutes, such as proteins and nucleic acid constructs. These are: i) intracellular axonal transport by the olfactory sensory neurons that project into the olfactory bulb of the brain; and ii) paracellular transport through clefts between olfactory epithelial cells in the nasal mucosa. *Trans*-neuronal (i.e., intracellular) transport requires that the drug first be endocytosed by the olfactory neurons and then transported down the axon. The typically rapid appearance of intranasally administered peptides or proteins in the brain suggests that they are unlikely to have been transported by this route. Rather, paracellular transport (movement between cells), which occurs more rapidly, may account for a greater percentage of the brain uptake of proteins observed within minutes to hours of intranasal delivery [74,75]. Once in the brain, distinct anatomical pathways seem to exist for the transport of proteins throughout the tissue and may reach as far as the brain stem.

Small, lipophilic drugs are generally transported most efficiently by the intranasal route, and a number of such drugs have been introduced into the pharmaceutical market, including sumatriptan, zolmitriptan, ergotamine and butorphanol [76]. However, the intranasal delivery of peptides, proteins and genes is also attracting pharmaceutical interest

as evidence accumulates for their rapid, direct nose-to-brain transport in both humans and animals [77,78]. The uptake of peptides and proteins from the nose has been shown to be quite rapid and most likely involves the paracellular route. In one study, uptake of three neuropeptides (melanocortin 4 – 10, vasopressin, and insulin) was detected in the CSF of human subjects within 80 min of intranasal administration [79]. In another study, intranasal administration of insulin to humans yielded concentrations in the brain that were > 400-fold higher than those achieved by the subcutaneous route, and uptake was again very rapid, that is within 10 min after administration [80]. In animal studies, a number of peptide molecules and growth factors have been successfully delivered to the brain by the nasal route, and many have been shown to provide neuroprotection in various disease models [69,70,80,81]. For instance, insulin-like growth factor-1 (IGF-1) given intranasally to rats has been shown to bypass the BBB, enter the brain and distribute along the olfactory and trigeminal pathways. Detectable levels were achieved within 30 min in rostral brain areas (including olfactory bulb and tubercle, frontal and motor cortex, and striatum) as well as caudal and ventral structures (including midbrain) [72,73]. It is important to note that the protein was delivered in quantities sufficient to activate signaling pathways in brain regions known to express the IGF-1 receptor. Intranasal IGF-1 also exerted neuroprotective effects in the rat middle cerebral artery occlusion model of stroke, where it reduced infarct size and improved neurological function [82]. NGF has also been administered intranasally to rats and was found in the brain within an hour of delivery. It also reversed the cognitive deficits in an anti-NGF transgenic mouse model of AD [70]. Basic fibroblast growth factor given intranasally protected against motor deficits and the loss of striatal dopamine in the mouse MPTP model of PD, and it also increased neurogenesis in the subventricular zone of mice [83]. As a final example, nasal administration of 'activity-derived neurotrophic factor' and a related neurotrophic peptide protected against the short-term memory loss induced by the cholinergic neurotoxin AF64A [81].

Despite significant promise, intranasal delivery of CNS therapeutics for human use is highly controversial and has some clear limitations. Although the olfactory route does not have a BBB, tight junctions are present in the epithelial layer of the nasal cavity, as are many metabolic enzymes, just as those present at the BBB [67]. Additionally, the anatomy of the intranasal route is significantly different between rodent models and actual human administration [84]. The small volumes that can be administered in each nostril (typically 10 – 25 µL in rodents and < 100 µL in humans), the short residence time of the drug at the nasal mucosa and poor permeability across mucus and tissue layers are also important factors to consider in nasal delivery [85]. Due to the small amount of drug that actually enters the CNS through this route (bioavailability < 1%), extremely potent drugs, genes and siRNA are the only options for this route of administration. However, the advantage of a routine 'patient-friendly' route of administration is an important issue for consideration in intranasal delivery.

In order to avoid systemic effects of these drugs, it would be desirable for the drugs to be broken down in the digestive tract, so that those not absorbed in the nose will pass down and be eliminated from the body. Another area of debate is over the vast difference in the nasal anatomy between rodents and humans. This has drastic implications, as the anatomical differences can cause a treatment that shows efficacy in rodents to be ineffective in human administration. Additional preclinical evidence, especially relevant animal models, such as non-human primates, will be necessary to further understand the benefits and limitations of intranasal delivery, especially for large molecular proteins, peptides and nucleic acid constructs. Additionally, advanced delivery strategies, such as nanoparticles, dry powders, or mucoadhesive gels, which can enhance residence time at the nasal mucosa, decrease premature drug degradation, facilitate nose-to-brain transport and increase availability of drugs in target tissues and cells are important formulation considerations that will have to be examined.

4.5 Nanotechnology for CNS delivery

Nanotechnology has an important role to play in the development of pharmaceutical products by affecting the drug biodistribution and pharmacokinetics, as well as enhancing bioavailability in targeted sites upon oral and systemic administration [86]. For CNS delivery, nano-sized carriers are designed to effectively package the therapeutic or imaging payload and enhance transport across the BBB. Through the use of liposomes, emulsions, solid–lipid nanoparticles and polymeric nanoparticles, drugs can be delivered to the CNS while avoiding extensive systemic distribution. These systems also allow a variety of agents to be concentrated within the nanocarrier. These carriers can then be absorbed orally to allow for a greater oral bioavailability when dealing with challenging drugs. It is important to note, however, that some of the components of these delivery systems, such as polysorbate 80, have been shown to disrupt different aspects of the BBB. It must also be noted that the elimination and toxicity of many nanoparticles used in drug delivery confounds the question of treatment. Each delivery system must undergo extensive toxicity studies. These nanocarriers may not only have cellular effects, but due to the targeted nature of CNS delivery, the effects of accumulation in the CNS tissues must be critically evaluated to ensure limited or no toxicity. Several nanotechnology strategies used for CNS delivery are illustrated in **Figure 3**.

4.5.1 Liposomes

Liposomes are a well-characterized drug delivery system. Briefly, liposomes resemble the plasma membranes that make up cells. Traditionally, they are composed of

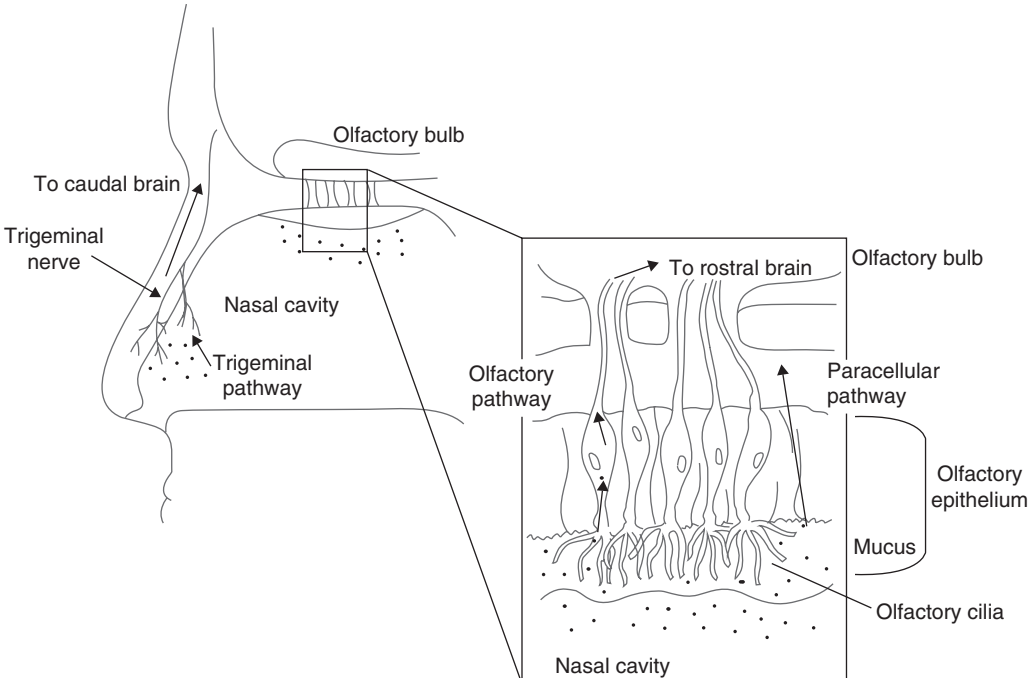

Figure 2. Schematic illustration of the transport pathways into the brain following intranasal delivery.

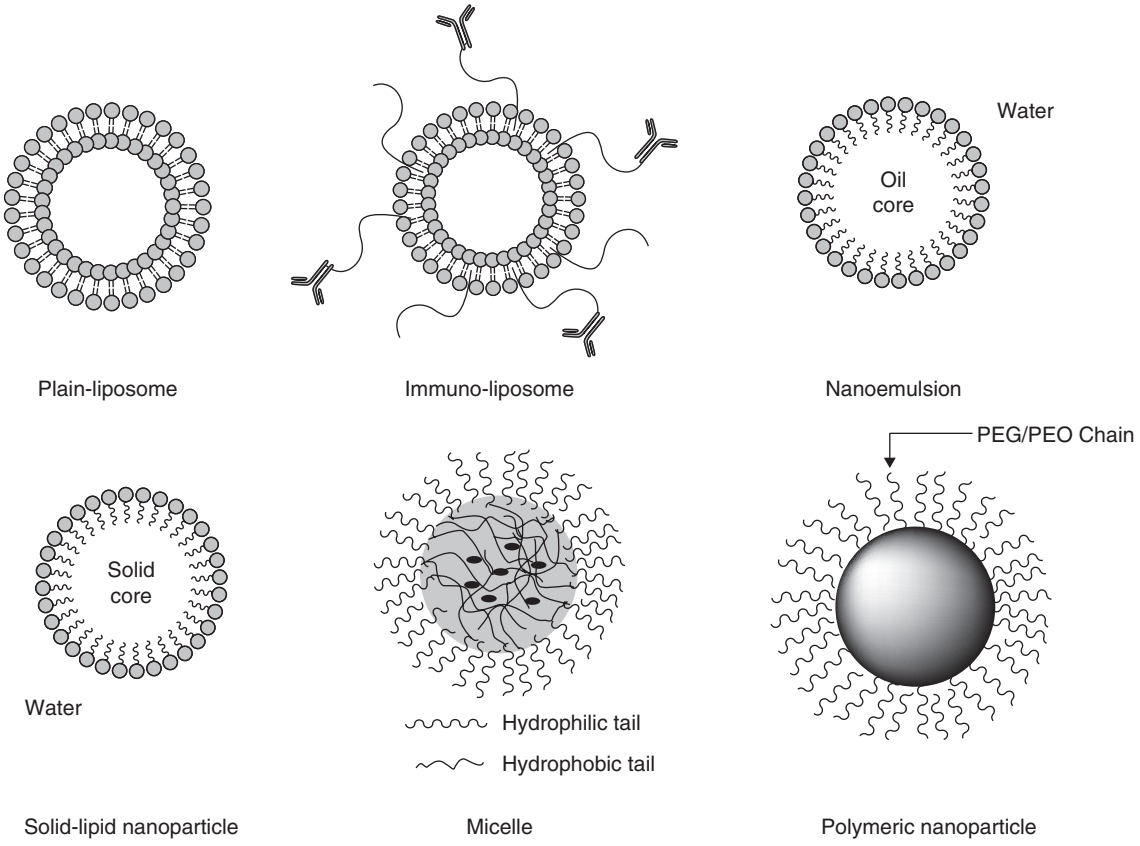

Figure 3. Illustrative examples of selected nano-sized delivery systems that have been examined for systemic delivery of drugs and genes into the brain.

phospholipids whose fatty acid tails interact, stabilizing the formation of a membrane. Liposomes have been used for a variety of pharmaceutical delivery systems; however, unmodified liposomes have had minimal success in the treatment of CNS diseases. Immunoliposomes have been successfully used to traffic many compounds into the CNS [87]. By grafting an antibody to the surface of the liposome, the liposome can be targeted to the endothelial cells of the BBB, increasing CNS absorption of the liposome [88]. This allows for enhanced delivery of many drug molecules to the CNS that would otherwise been unable to be taken up. Another advantage of using immunoliposomes is the specificity of distribution. Because the antibody is targeted to the BBB, the liposome is preferentially taken up in the CNS. This allows for decreased side effects due to non-specific distribution. Even though the immunoliposome is targeted to the CNS for preferential uptake, a major portion of the dose is still taken up by off-target sites where it can have adverse effects. In order to achieve safer CNS targeting, it would be ideal to use a CNS-specific drug. When taken up by the CNS it would have its effects, but in other tissues it would remain inert. It has been shown that immunoliposomes can successfully deliver antisense RNA to the CNS [89]. By grafting a CNS-specific antibody onto the liposome, it is preferentially taken up by the CNS, where the drug can have its intended effects. It has been shown that a variety of natural ligands can be used to traffic drugs into the CNS. The natural ligands act as 'molecular Trojan horses' which carry drug across the BBB [90]. This approach has also been applied to chimeric proteins where the protein of interest is trafficked into the CNS by the natural BBB penetration ability of the other half of the protein [62].

4.5.2 *Nanoemulsions*

In a typical pharmaceutical emulsion, either oil is dispersed in water (oil-in-water emulsion; O/W) or water is dispersed in oil (water-in-oil emulsion; W/O) through the use of surface-active agents, also known as surfactants [91]. The surfactant systems allow for the oil and water phases to be combined into a unified delivery system, which would otherwise separate. Nanoemulsions are an emulsion system where the diameter of the inner phase is reduced to the nanometer scale (typically 100 – 200 nm). Nanoemulsions can facilitate oral absorption of drugs, due to the ability of the O/W emulsion to readily disperse in water [92]. The use of nanoemulsion systems has also been shown to increase CNS penetration of drugs. One example of this approach is the use of nanoemulsion systems for the CNS delivery of saquinavir. It was shown that the use of a nanoemulsion to administer drugs caused an increase in brain uptake of saquinavir [92]. This has been attributed to the use of oils rich in omega-3 polyunsaturated fatty acids for the formulation of the nanoemulsion, as omega-3 fatty acids are preferentially transported into the brain [93]. Through advances in the understanding of emulsion distribution, a novel system may be able to penetrate the BBB as an effective treatment strategy.

4.5.3 *Solid–lipid nanoparticles*

Solid–lipid nanoparticles (SLNs) are surfactant-stabilized aqueous colloidal dispersions of lipid nanoparticles that solidify upon cooling. These are a major alternative to the polymeric nanoparticles, and have certain advantages over the polymeric systems. SLNs resemble, in many respects, O/W emulsions. They contain a lipid phase dispersed in an aqueous environment. This allows SLNs to be composed of biocompatible lipids, and large-scale production of these systems is feasible [94]. These lipids can freely carry hydrophobic compounds in the lipid phase of the nanoparticles. Poly(ethylene glycol)-modified SLNs have been shown to penetrate the BBB and allow for greater delivery of drug to the CNS [95]. Thiamine-coated nanoparticles have shown increased uptake in the CNS for the potential treatment of malignant gliomas [96]. Through their stability and ease of manufacture, SLNs are a promising area for drug delivery to the CNS.

4.5.4 *Polymeric nanoparticles*

Polymeric nanoparticles are solid colloidal particles created from polymeric systems. These nanoparticles are made from biocompatible polymers that encapsulate or adsorb drugs for prolonged release. It has been shown that poly(butylcyanoacrylate) nanoparticles coated with polysorbate 80 are able to deliver drugs to the brain [97]. The mechanism of polysorbate 80-mediated BBB transport is debated, and as polysorbate 80 has been shown to disrupt the BBB, this may contribute to the ability of these nanoparticles to penetrate the BBB. The role of polysorbate 80 in BBB penetration has also been examined in polylactic acid nanoparticles. It was shown that polysorbate 80 modification was critical for transport of these polymeric nanoparticles across the BBB [98]. Although the polysorbate 80 is required for BBB penetration of these systems, it may still provide an interesting approach to treatment of neurodegenerative disease. **Table 3** summarizes some of the nanotechnology applications in CNS delivery.

5. Conclusions

There is a tremendous need to develop better therapeutics for neurodegenerative diseases that can modify disease progression rather than just treat symptoms. Although biological macromolecules such as antibodies, growth factors and siRNA have shown tremendous promise in arresting neurodegenerative disease progression and, in some cases, rescuing neurons in pre-clinical models, translation of these laboratory studies into clinical practice is very difficult due to the lack of availability of these agents in the brain following systemic delivery. Several different strategies are addressed to enhance the availability of biological

Table 3. Selected examples of nanoparticle systems used for CNS drug delivery.

Drug	Formulation	Proposed treatment	Ref.
Cisplatin	Liposome	Cancer	[109]
FITC	Liposome	Proof of concept	[110]
Daunomycin	Liposome	Cancer	[88]
NGF	Liposome	Neurodegeneration	[111]
Irinotecan	Liposome	Cancer	[112]
Saquinavir	Emulsion	Antiviral	[92]
Paclitaxel	Solid lipid nanoparticle	Cancer	[113]
Thiamine	Solid lipid nanoparticle	Proof of concept study	[96]
Etoposide	Solid lipid nanoparticle	Cancer	[114]
Camptothecin	Solid lipid nanoparticle	Cancer	[115]
Ritonavir	Polymeric nanoparticle	Anti-retroviral	[116]
Zidovudine/lamivudine	Polymeric nanoparticle	Anti-retroviral	[117]
Doxorubicin	Polymeric nanoparticle	Cancer	[118]
Loperamide	Polymeric nanoparticle	Analgesia	[119]
Tetramethylrhodamine	Polymeric nanoparticle	Proof of concept	[120]
Reporter plasmid	Dendrimer	Gene therapy	[121]

macromolecules in the brain including invasive (e.g., intracranial and temporary disruption of BBB) to non-invasive (e.g., intranasal and use of nanotechnology-based delivery systems) strategies. In each case, the advantages and disadvantages of the approach and relevant examples from contemporary scientific literature are presented. It is clear that delivery strategies will have to be integrated early in the development of CNS therapeutics. In many instances, a combination of the delivery strategies discussed in this review would be beneficial and may allow for greater CNS availability of the therapeutic agent.

6. Expert opinion

The number of patients with chronic neurodegenerative diseases such as AD, PD and HD is projected to increase significantly in the future due to the longer lifespan and the aging of the Western population. With this demographic change, there is an urgent need to develop novel therapeutics for neurodegenerative diseases that affect the disease progression, rather than simply treating the symptoms. Additionally, these molecularly targeted therapeutic agents also promise to have fewer side effects as compared to conventional low molecular weight drugs. Over 98% of low molecular weight and 100% of proteins and nucleic acid therapeutics currently investigated, however, are not able to cross the BBB and afford sufficient availability in the CNS upon systemic administration [99]. Lack of availability is often due to dilution effects in the systemic circulation, premature degradation or metabolism, and, most importantly, poor permeability through the tight cellular junctions of the BBB. As we achieve greater understanding of disease mechanisms and molecular pathways that can be interrupted for therapeutic purposes in neurodegenerative diseases, the need for effective delivery in the CNS will be even more pronounced.

Current delivery strategies that are used in the clinic or those in experimental stages can be divided into either invasive or non-invasive approaches. Direct intracranial administration using catheters or transient disruption of the BBB using hyperosmotic solutions, such as mannitol, are examples of invasive strategies that have shown limited success in the clinic. Unfortunately, intracranial delivery carries significant surgical risks, including thrombo-embolic complications and infections, and may not be suitable for chronic neurodegenerative therapy. The use of hyperosmotic mannitol infusions into the carotid artery also leads to significant pain and debilitation, and it can only be practiced under extreme care in a clinical setting. Non-invasive delivery strategies, including chemical structure modification and concurrent administration of efflux transport inhibitors, have a role to play in the delivery of low molecular weight therapeutic agents. However, these strategies are also not practical for chronic administration of biological macromolecules. In contrast, based on the preliminary work of Frey *et al.*, [72–73,100-102] the intranasal route of administration shows significant promise in CNS delivery of potent biological macromolecules, such as growth factors. Despite some limiting issues, such as small volume of administration, short residence time, enzyme-mediated degradation, poor permeability across nasal mucosal surface and potential for toxicity, intranasal delivery can still achieve significantly greater bioavailability

for proteins than any other approach, excluding direct intracranial administration. The intranasal route of administration also affords an opportunity for repeated self-administration, which will lead to higher patient compliance. Another area of promise is in the development of nanotechnology-based delivery systems with potential to enhance availability in the CNS. These systems have the potential to enable delivery of biopharmaceuticals through the BBB, as the endothelial cells at the BBB can take them up more readily than the biopharmaceuticals themselves. Surface modification can also overcome the negatively charged cell surface of the BBB that attributes to poor biopharmaceutical delivery to the CNS. Although several different types of nanoparticles, such as liposomes, nanoemulsions and solid–lipid nanoparticles, have been examined for delivery of drugs and image contrast enhancers in the brain, the majority of these studies were carried out in rodent models. In order to further these experimental strategies beyond the confines of the laboratory, the delivery efficiency and safety of nanoparticles will have to be critically evaluated in other relevant animal models, such as non-human primates.

Due to the immediate and profound need for effective therapy in neurodegenerative diseases, the development of drug delivery strategies is an area of immense importance. Success in this area will clearly stem from the work of academic and industrial scientists, whose passion and creativity will yield transformative solutions in the CNS delivery challenge.

Declaration of interest

This paper does not reflect any financial, commercial, or other relationship between the authors and any other party.

Bibliography

Papers of special note have been highlighted as either of interest (•) or of considerable interest (••) to readers.

1. Federoff HJ. Novel targets for CNS gene therapy. Gene Ther 1999;6(12):1907-8
2. Pardridge WM. Why is the global CNS pharmaceutical market so under-penetrated? Drug Discov Today 2002;7(1):5-7
3. Tiwari SB, Amiji MM. A review of nanocarrier-based CNS delivery systems. Curr Drug Deliv 2006;3(2):219-32
4. Azzouz M, Ralph S, Wong LF, et al. Neuroprotection in a rat Parkinson model by GDNF gene therapy using EIAV vector. Neuroreport 2004;15(6):985-90
5. Bickel U, Yoshikawa T, Pardridge WM. Delivery of peptides and proteins through the blood–brain barrier. Adv Drug Deliv Rev 2001;46(1-3):247-79
6. Abdellatif AA, Pelt JL, Benton RL, et al. Gene delivery to the spinal cord: comparison between lentiviral, adenoviral, and retroviral vector delivery systems. J Neurosci Res 2006;84(3):553-67
7. Berry M, Barrett L, Seymour L, et al. Gene therapy for central nervous system repair. Curr Opin Mol Ther 2001;3(4):338-49
8. Costantini LC, Bakowska JC, Breakefield XO, Isacson O. Gene therapy in the CNS. Gene Ther 2000;7(2):93-109
9. Federoff HJ. CNS diseases amenable to gene therapy. Ernst Schering Res Found Workshop 2003;(43):117-58
10. Friedmann T. Gene therapy for disorders of the CNS. Gene Ther 1994;1(Suppl 1):S47-8
11. Taylor JP, Hardy J, Fischbeck KH. Toxic proteins in neurodegenerative disease. Science 2002;296:1991-5
12. Buckner RL, Snyder AZ, Shannon BJ, et al. Molecular, structural, and functional characterization of alzheimer's disease: evidence for a relationship between default activity, amyloid, and memory. J Neurosci 2005;25:7709-17
13. Alavijeh MS, Chishty M, Qaiser MZ, Palmer AM. Drug metabolism and pharmacokinetics, the blood–brain barrier, and central nervous system drug discovery. NeuroRx 2005;2(4):554-71
14. Hebert LE, Scherr PA, Bienias JL, et al. Alzheimer disease in the US population: prevalence estimates using the 2000 census. Arch Neurol 2003;60:1119-22
15. Melnikova I. Therapies for Alzheimer's disease. Nat Rev Drug Discov 2007;6(5):341-2
16. Schneider LS, Tariot PN, Dagerman KS, et al. Effectiveness of atypical antipsychotic drugs in patients with Alzheimer's disease. N Engl J Med 2006;355(15):1525-38
17. Lim G. The curry spice curcumin reduces oxidative damage and amyloid pathology in an Alzheimer transgenic mouse. J Neurosci 2001;21(21):8370-7
18. Lim GP, Calon F, Morihara T, et al. A diet enriched with the omega-3 fatty acid docosahexaenoic acid reduces amyloid burden in an aged Alzheimer mouse model. J Neurosci 2005;25:3032-40
19. Tobinick E, Gross H. Rapid cognitive improvement in Alzheimer's disease following perispinal etanercept administration. J Neuroinflamm 2008;5(1):2

• **This paper shows that etanercept has potential, if an acceptable delivery strategy can be employed.**

20. Tuszynski MH. Growth-factor gene therapy for neurodegenerative disorders. Lancet Neurology 2002;1(1):51-7
21. Tuszynski MH, Thal L, Pay M, et al. A phase 1 clinical trial of nerve growth factor gene therapy for Alzheimer disease. Nat Med 2005;11(5):551-5
22. Opar A. Mixed results for disease-modification strategies for Alzheimer's disease. Nat Rev Drug Discov 2008;7(9):717-8
23. Weintraub D, Comella CL, Horn S. Parkinson's disease – Part 1: Pathophysiology, symptoms, burden, diagnosis, and assessment. Am J Manag Care 2008;14:S40-8
24. Weintraub D, Comella CL, Horn S. Parkinson's disease – Part 2: Treatment of motor symptoms. Am J Manag Care 2008;14:S49-58
25. Kordower JH, Emborg ME, Bloch J, et al. Neurodegeneration prevented by lentiviral vector delivery of GDNF in primate models of Parkinson's disease. Science 2000;290(5492):767-73
26. Manfredsson FP, Lewin AS, Mandel RJ. RNA knockdown as a potential therapeutic

strategy in Parkinson's disease. Gene Ther 2005;13(6):517-24

27. Walker FO. Huntington's disease. Lancet 2007;369:218-28
28. Imarisio S, Carmichael J, Korolchuk V, et al. Huntington's disease: from pathology and genetics to potential therapies. Biochem J 2008;412:191-209
29. Adam OR, Jankovic J. Symptomatic treatment of Huntington Disease. Neurotherapeutics 2008;5:181-97
30. Machida Y, Okada T, Kurosawa M, et al. rAAV-mediated shRNA ameliorated neuropathology in Huntington disease model mouse. Biochem Biophys Res Commun 2006;343(1):190-7
31. Pardridge WM. The blood–brain barrier: bottleneck in brain drug development. NeuroRx 2005;2:3–14
32. Ehrlich P. Das Sauerstoff-Bedurfniss des Organismus, eine farbanalytische Studie. Hirschwald, Berlin, 1885
33. Hawkins BT, Davis TP. The blood–brain barrier/neurovascular unit in health and disease. Pharmacol Rev 2005;57(2):173-85
34. Reese TS, Karnovsky MJ. Fine structural localization of a blood–brain barrier to exogenous peroxidase. J Cell Biol 1967;34(1):207-17
35. Abbott NJ, Ronnback L, Hansson E. Astrocyte-endothelial interactions at the blood–brain barrier. Nat Rev Neurosci 2006;7:41-53
36. Zlokovic B. The blood–brain barrier in health and chronic neurodegenerative disorders. Neuron 2008;57(2):178-201

• **This paper provides an excellent review of the BBB with regards to neurodegeneration.**

37. Ghersi-Egea JF, Minn A, Siest G. A new aspect of the protective functions of the blood–brain barrier: activities of four drug-metabolizing enzymes in isolated rat brain microvessels. Life Sci 1988;42(24):2515-23
38. Minn A, Ghersi-Egea JF, Perrin R, et al. Drug metabolizing enzymes in the brain and cerebral microvessels. Brain Res Rev 1991;16(1):65-82
39. Pardridge WM. Drug and gene delivery to the brain: the vascular route. Neuron 2002;36:555-8

• **This paper provides a concise overview of the pathways for drug delivery to the CNS from blood.**

40. Pardridge WM. Recent developments in peptide drug delivery to the brain. Pharmacol Toxicol 1992;71(1):3-10
41. Strasser JF, Fung LK, Eller S, et al. Distribution of 1,3-bis(2-chloroethyl)-1-nitrosourea and tracers in the rabbit brain after interstitial delivery by biodegradable polymer implants. J Pharmacol Exp Ther 1995;275(3):1647-55
42. Storm PB, Moriarity JL, Tyler B, et al. Polymer delivery of camptothecin against 9L gliosarcoma: release, distribution, and efficacy. J Neurooncol 2002;56(3):209-17
43. Ernerich DF, Plone M, Francis J, et al. Alleviation of behavioral deficits in aged rodents following implantation of encapsulated GDNF-producing fibroblasts. Brain Res 1996;736(1-2):99-110
44. Lang AE, Gill S, Patel NK, et al. Randomized controlled trial of intraputamenal glial cell line-derived neurotrophic factor infusion in Parkinson disease. Ann Neurol 2006;59(3):459
45. Hovland DN, Boyd RB, Butt MT, et al. Six-month continuous intraputamenal infusion toxicity study of recombinant methionyl human glial cell line-derived neurotrophic factor (r-metHuGDNF) in Rhesus monkeys. Toxicol Pathol 2007;35(5):676
46. Bloch J, Bachoud-Levi AC, Deglon N, et al. Neuroprotective gene therapy for Huntington's disease, using polymer-encapsulated cells engineered to secrete human ciliary neurotrophic factor: results of a phase I study. Hum Gene Ther 2004;15(10):968-75
47. Zünkeler B, Carson RE, Olson J, et al. Quantification and pharmacokinetics of blood–brain barrier disruption in humans. J Neurosurg 1996;85(6):1056-65
48. Wennberg R. The blood–brain barrier and bilirubin encephalopathy. Cell Mol Neurobiol 2000;20(1):97-109
49. Hanig JP, Morrison JM Jr, Krop S. Ethanol enhancement of blood–brain barrier permeability to catecholamines in chicks. Eur J Pharmacol 1972;18(1):79-82
50. Broadwell RD, Salcman M, Kaplan RS. Morphologic effect of dimethyl sulfoxide on the blood–brain barrier. Science 1982;217(4555):164-6
51. Dudeja PK, Anderson KM, Harris JS, et al. Reversal of multidrug resistance phenotype by surfactants: relationship to membrane lipid fluidity. Arch Biochem Biophys 1995;319(1):309-15
52. Bogman K, Erne-Brand F, Alsenz J, Drewe J. The role of surfactants in the reversal of active transport mediated by multidrug resistance proteins. J Pharm Sci 2003;92(6):1250-61

• **This article describes a major factor in some of the early successes of nanoparticle use in CNS delivery due to the surfactant interaction with efflux transporters.**

53. Schenk D, Barbour R, Dunn W, et al. Immunization with amyloid-ß attenuates Alzheimer's disease-like pathology in the PDAPP mouse. Nature 1999;400(6740):173-7
54. Raymond SB, Treat LH, Dewey JD, et al. Ultrasound enhanced delivery of molecular imaging and therapeutic agents in Alzheimer's disease mouse models. PLoS ONE 2008;3(5):e2175
55. Ferrer I, Rovira MB, Guerra MLS, et al. Neuropathology and pathogenesis of encephalitis following amyloid ß Immunization in Alzheimer's disease. Brain Pathol 2004;14(1):11-20
56. NCBI. PubChem Compound. cited; Available from: http://pubchem.ncbi.nlm.nih.gov/
57. Standaert DG, Young AB. Treatment of central nervous system degenerative disorders. Eleventh editon. In: Brunton LL, editor, Goodman and Gillman's Pharmacologic basis of therapeutics. New York: McGraw-Hill; 2006. p. 527-45
58. Martel CL, Mackic JB, Adams JD, et al. Transport of Dopamine at the blood–brain barrier of the guinea pig: inhibition by psychotropic drugs and nicotine. Pharm Res 1996;13(2):290-5
59. Kageyama T, Nakamura M, Matsuo A, et al. The 4F2hc/LAT1 complex transports L-DOPA across the blood-brain barrier. Brain Res 2000;879(1-2):115-21
60. Tsuji A, Tamai I. Carrier-mediated or specialized transport of drugs across the blood–brain barrier. Adv Drug Deliv Rev 1999;36(2-3):277-90
61. Kumar P, Wu H, McBride JL, et al. Transvascular delivery of small interfering RNA to the central nervous system. Nature 2007;448:39-43

• **This paper describes a novel delivery strategy for siRNA to the CNS.**

62. Pardridge WM. Molecular Trojan horses for blood–brain barrier drug delivery. Curr Opin Pharm 2006;6:494-500
63. Bendayan R, Lee G, Bendayan M. Functional expression and localization of

P-glycoprotein at the blood brain barrier. Microsc Res Tech 2002;57(5):365-80

64. Sadeque AJ, Wandel C, He H, et al. Increased drug delivery to the brain by P-glycoprotein inhibition. Clin Pharm Ther 2000;68:231-7

65. Bodor N, Prokai L, Wu WM, et al. A strategy for delivering peptides into the central nervous system by sequential metabolism. Science 1992;257(5077):1698-700

• **This article provided one of the foundations for the 'lock-in' strategy to deliver drugs to the CNS.**

66. Wu J, Yoon SH, Wu WM, Bodor N. Synthesis and biological evaluations of brain-targeted chemical delivery systems of [Nva2]-TRH. J Pharm Pharmacol 2002;54(7):945-50

67. Graff CL, Pollack GM. Nasal drug administration: potential for targeted central nervous system delivery. J Pharm Sci 2005;94:1187-95

68. Tirucherai GS, Yang C, Mitra AK. Prodrugs in nasal drug delivery. Expert Opin Biol Ther 2001;1(1):49-66

69. Bagger MA, Bechgaard E. The potential of nasal application for delivery to the central brain – a microdialysis study of fluorescein in rats. Eur J Pharm Sci 2004;21(2-3):235-42

70. De Rosa R, Garcia AA, Braschi C, et al. Intranasal administration of nerve growth factor (NGF) rescues recognition memory deficits in AD11 anti-NGF transgenic mice. Proc Natl Acad Sci USA 2005;102(10):3811-6

71. Laursen T, Grandjean B, Jorgensen JO, Christiansen JS. Bioavailability and bioactivity of three different doses of nasal growth hormone (GH) administered to GH-deficient patients: comparison with intravenous and subcutaneous administration. Eur J Endocrinol 1996;135(3):309-15

72. Liu XF, Fawcett JR, Thorne RG, et al. Intranasal administration of insulin-like growth factor-I bypasses the blood–brain barrier and protects against focal cerebral ischemic damage. J Neurol Sci 2001;187(1-2):91-7

• **This provided evidence for the transport of larger molecules through the intranasal route.**

73. Liu XF, Fawcett JR, Thorne RG, Frey WH 2nd. Non-invasive intranasal insulin-like growth factor-I reduces infarct volume and improves neurologic function in rats following middle cerebral artery occlusion. Neurosci Lett 2001;308(2):91-4

74. Vyas TK, Shahiwala A, Marathe S, Misra A. Intranasal drug delivery for brain targeting. Curr Drug Deliv 2005;2(2):165-75

75. Lawrence D. Intranasal delivery could be used to administer drugs directly to the brain. Lancet 2002;359(9318):1674

76. Vyas TK, Babbar AK, Sharma RK, et al. Preliminary brain-targeting studies on intranasal mucoadhesive microemulsions of sumatriptan. AAPS PharmSciTech 2006;7(1):E8

77. Illum L. Is nose-to-brain transport of drugs in man a reality? J Pharm Pharmacol 2004;56(1):3-17

78. Invitti C, Fatti L, Camboni MG, et al. Effect of chronic treatment with octreotide nasal powder on serum levels of growth hormone, insulin-like growth factor I, insulin-like growth factor binding proteins 1 and 3 in acromegalic patients. J Endocrinol Invest 1996;19(8):548-55

79. Fehm HL, Perras B, Smolnik R, et al. Manipulating neuropeptidergic pathways in humans: a novel approach to neuropharmacology? Eur J Pharmacol 2000;405(1-3):43-54

80. Haruta S, Hanafusa T, Fukase H, et al. An effective absorption behavior of insulin for diabetic treatment following intranasal delivery using porous spherical calcium carbonate in monkeys and healthy human volunteers. Diabetes Technol Ther 2003;5(1):1-9

81. Gozes I, Giladi E, Pinhasov A, et al. Activity-dependent neurotrophic factor: intranasal administration of femtomolar-acting peptides improve performance in a water maze. J Pharmacol Exp Ther 2000;293(3):1091-8

82. Zhao HM, Liu XF, Mao XW, Chen CF. Intranasal delivery of nerve growth factor to protect the central nervous system against acute cerebral infarction. Chin Med Sci J 2004;19(4):257-61

83. Shi J, Perry G, Berridge MS, et al. Labeling of cerebral amyloid beta deposits in vivo using intranasal basic fibroblast growth factor and serum amyloid P component in mice. J Nucl Med 2002;43(8):1044-51

84. Merkus FWHM, van den Berg MP. Can nasal drug delivery bypass the blood–brain barrier? Drugs R D 2007;8(3):133-44

•• **This review examined the differences between intranasal delivery in animals and humans. This gives an anatomical basis and experimental data to suggest that intranasal delivery in humans may be more challenging than previously thought**

85. Vyas TK, Tiwari SB, Amiji MM. Formulation and physiological factors influencing CNS delivery upon intranasal administration. Crit Rev Ther Drug Carrier Syst 2006;23(4):319-47

86. Devalapally H, Chakilam A, Amiji MM. Role of nanotechnology in pharmaceutical product development. J Pharm Sci 2007;96(10):2547-65

87. Tiwari SB, Amiji MM. A review of nanocarrier-based CNS delivery systems. Curr Drug Deliv 2006;3:219-32

88. Huwyler J, Wu D, Pardridge WM. Brain drug delivery of small molecules using immunoliposomes. Proc Natl Acad Sci 1996;93:14164-9

89. Zhang Y, Zhu C, Pardridge WM. Antisense gene therapy of brain cancer with an artificial virus gene delivery system. Mol Ther 2002;6(1):67-72

90. Pardridge WM. Drug and gene targeting to the brain with molecular Trojan horses. Nat Rev Drug Discov 2002;1(2):131-9

91. McClements DJ, Decker EA, Weiss J. Emulsion-based delivery systems for lipophilic bioactive components. J Food Sci 2007;72:R109-24

92. Vyas TK, Shahiwala A, Amiji MM. Improved oral bioavailability and brain transport of Saquinavir upon administration in novel nanoemulsion formulations. Int J Pharm 2008;347:93-101

93. Bernoud N, Fenart L, Benistant C, et al. Astrocytes are mainly responsible for the polyunsaturated fatty acid enrichment in blood-brain barrier endothelial cells in vitro. J Lipid Res 1998;39:1816-24

94. Müller RH, Mäder K, Gohla S. Solid lipid nanoparticles (SLN) for controlled drug delivery – a review of the state of the art. Eur J Pharm Biopharm 2000;50(1):161-77

95. Zara GP, Cavalli R, Bargoni A, et al. Intravenous administration to rabbits of non-stealth and stealth doxorubicin-loaded solid lipid nanoparticles at increasing concentrations of stealth agent: pharmacokinetics and distribution of doxorubicin in brain and other tissues. J Drug Target 2002;10(4):327-35

96. Lockman PR, Oyewumi MO, Koziara JM, et al. Brain uptake of thiamine-coated nanoparticles. J Control Release 2003;93(3):271-82

97. Kreuter J, Alyautdin RN, Kharkevich DA, Ivanov AA. Passage of peptides through the blood–brain barrier with colloidal polymer particles (nanoparticles). Brain Res 1995;674(1):171-4

98. Sun W, Xie C, Wang H, Hu Y. Specific role of polysorbate 80 coating on the targeting of nanoparticles to the brain. Biomaterials 2004;25(15):3065-71

99. Pardridge WM. The blood–brain barrier: bottleneck in brain drug development. NeuroRx 2005;2(1):3-14

100. Hanson LR, Frey WH 2nd. Strategies for intranasal delivery of therapeutics for the prevention and treatment of neuroAIDS. J Neuroimmune Pharmacol 2007;2(1):81-6

101. Thorne RG, Frey WH 2nd. Delivery of neurotrophic factors to the central nervous system: pharmacokinetic considerations. Clin Pharmacokinet 2001;40(12):907-46

102. Thorne RG, Pronk GJ, Padmanabhan V, Frey WH 2nd. Delivery of insulin-like growth factor-I to the rat brain and spinal cord along olfactory and trigeminal pathways following intranasal administration. Neuroscience 2004;127(2):481-96

103. Dorsey ER, Constantinescu R, Thompson JP, et al. Projected number of people with Parkinson disease in the most populous nations, 2005 through 2030. Neurology 2007;68(5):384-6

104. Steinman MDL. Multiple Sclerosis: a coordinated immunological attack against myelin in the central nervous system. Cell 1996;85(3):299-302

105. Preux PM, Druet-Cabanac M, Couratier P, et al. Estimation of the amyotrophic lateral sclerosis incidence by capture–recapture method in the Limousin region of France. J Clin Epidemiol 2000;53(10):1025-9

106. Sun M, Kong L, Wang X, et al. Comparison of the capability of GDNF, BDNF, or both, to protect nigrostriatal neurons in a rat model of Parkinson's disease. Brain Res 2005;1052(2):119-29

107. Aebischer P, Ridet JL. Recombinant proteins for neurodegenerative diseases: the delivery issue. Trends Neurosci 2001;24(9):533-40

108. Kordower JH, Isacson O, Emerich DF. Cellular delivery of trophic factors for the treatment of Huntington's disease: is neuroprotection possible? Exp Neurol 1999;159:4-20

109. Shibata S, Ochi A, Mori K. Liposomes as carriers of cisplatin into the central nervous system–experiments with 9L gliomas in rats. Neurol Med Chir (Tokyo) 1990;30(4):242-5

110. Yamada K, Moriguchi A, Morishita R, et al. Efficient oligonucleotide delivery using the HVJ-liposome method in the central nervous system. Am J Physiol Regul Integr Comp Physiol 1996;271(5):R1212-20

111. Teresa Girão da Cruz M, Cardoso ALC, de Almeida LP, et al. Tf-lipoplex-mediated NGF gene transfer to the CNS: neuronal protection and recovery in an excitotoxic model of brain injury. Gene Ther 2005;12(16):1242-52

112. Krauze MT, Noble CO, Kawaguchi T, et al. Convection-enhanced delivery of nanoliposomal CPT-11 (irinotecan) and PEGylated liposomal doxorubicin (Doxil) in rodent intracranial brain tumor xenografts. Neurooncol 2007;9(4):393-403

113. Koziara JM, Lockman PR, Allen DD, Mumper RJ. Paclitaxel nanoparticles for the potential treatment of brain tumors. J Control Release 2004;99(2):259-69

114. Reddy L, Sharma R, Chuttani K, et al. Etoposide-incorporated tripalmitin nanoparticles with different surface charge: formulation, characterization, radiolabeling, and biodistribution studies. AAPS Journal 2004;6(3):55-64

115. Yang SC, Lu LF, Cai Y, et al. Body distribution in mice of intravenously injected camptothecin solid lipid nanoparticles and targeting effect on brain. J Control Release 1999;59(3):299-307

116. Rao KS, Reddy MK, Horning JL, Labhasetwar V. TAT-conjugated nanoparticles for the CNS delivery of anti-HIV drugs. Biomaterials 2008;29(33):4429-38

• **This shows a strategy for using endogenous transporters to traffic encapsulated drug payload.**

117. Kuo YC, Chen HH. Effect of nanoparticulate polybutylcyanoacrylate and methylmethacrylate-sulfopropylmethacrylate on the permeability of zidovudine and lamivudine across the in vitro blood–brain barrier. Int J Pharm 2006;327:160-9

118. Ambruosi A, Khalansky AS, Yamamoto H, et al. Biodistribution of polysorbate 80-coated doxorubicin-loaded [14 C]-poly(butyl cyanoacrylate) nanoparticles after intravenous administration to glioblastoma-bearing rats. J Drug Target 2006;14(2):97-105

119. Tosi G, Costantino L, Rivasi F, et al. Targeting the central nervous system: In vivo experiments with peptide-derivatized nanoparticles loaded with Loperamide and Rhodamine-123. J Control Release 2007;122(1):1-9

120. Costantino L, Gandolfi F, Tosi G, et al. Peptide-derivatized biodegradable nanoparticles able to cross the blood–brain barrier. J Control Release 2005;108(1):84-96

121. Huang RQ, Qu YH, Ke WL, et al. Efficient gene delivery targeted to the brain using a transferrin-conjugated polyethyleneglycol-modified polyamidoamine dendrimer. FASEB J 2007;21(4):1117-25

Affiliation

Thomas M Barchet & Mansoor M Amiji†
†Author for correspondence
Northeastern University, School of Pharmacy, Department of Pharmaceutical Sciences, 110 Mugar Life Sciences Building, Boston, Massachussets, MA 02115, USA
Tel: +1 617 373 3137; Fax +1 617 373 8886;
E-mail: m.amiji@neu.edu