Delivering Therapies to the Brain: A Brief Review of Current Strategies for Huntington's Disease

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Introduction

Most potentially neuroprotective therapies now being explored for Huntington's disease (HD) and other neurodegenerative disorders will need the assistance of a drug delivery system to breach the blood–brain barrier (BBB) and to reach their targets in the human brain. Outside of cancer therapeutics, however, no drug delivery system has been shown safe and effective for delivering chronic treatments to the brain. A successful therapy for HD will likely have to achieve three crucial milestones: (1) crossing the BBB into the brain, (2) reaching the specific cells to which it is directed, and (3) performing its ameliorative function there. Further, any therapeutic must have a safety profile that supports chronic dosing and demonstrate success in a relevant HD preclinical model.

Drug Delivery Routes

Direct delivery to the brain

In HD patients, mutant huntingtin (*HTT*) is present in every cell of the body. One key question, then, is which cells should be targeted for the best chance of eliciting a therapeutic effect. Most discussions so far have focused on the striatum, with the hope that reducing the levels of *HTT* mRNA will rescue striatal neurons and motor signs.

Isis Pharmaceuticals (Carlsbad, CA) is using intrathecally delivered antisense oligonucleotides (ASOs) to reduce *HTT* levels in the brain, while a collaboration between Alnylam Pharmaceuticals (Cambridge, MA) and Medtronic (Minneapolis, MN) is pursuing the same goal by using small interfering RNA (siRNA) delivered intraparenchymally (Smith et al., 2006; Akinc et al, 2010). A proprietary Medtronic infusion system uses an implantable, battery-powered drug-infusion pump to deliver siRNA to the striatum, using convection-enhanced delivery for a wider distribution of the siRNA (Dickinson et al., 2010; Sah and Aronin, 2011).

At this point, neither Isis's antisense oligonucleotides nor Alnylam's siRNA is specific to mutant *HTT*; in both cases, wild-type *HTT* mRNA is also reduced. Since *HTT* participates in many cellular processes, excessive loss of *HTT* may be toxic, thus raising concerns about the therapeutic window for such treatment.

Intranasal delivery

Delivering drugs via the nasal passages could provide a noninvasive way to bypass the BBB and avoid toxicity due to systemic administration (Dhuria et al., 2010). In the olfactory epithelium, primary sensory neurons regenerate every 30 days or so (a unique property among neurons), which means that tight junctions are lacking in areas of immature cells. Work in animal models and humans suggests that a variety of particles — e.g., small molecules, neurotrophins, chemotherapeutics, oligonucleotides, stem cells — could be delivered to the brain in this way.

The highest concentration of particles delivered through the nose ends up in the olfactory bulb, medulla, and brainstem (at the entry point of the trigeminal nerves); however, widespread delivery to the striatum and cortex also occurs. Leah Hanson and her colleagues have shown that cargo distribution appears similar among different types of molecules. The fraction of the total dose delivered to brain, however, is highly variable and amounts to only 2–4%.

In rodents, cargo appears in the brain within 5 to 10 min, peaking after 30 min after intranasal administration. How the drug is delivered (for example, where it is placed on the mucoepithelium, whether the animal is anaesthetized or not) can change that time course. Some molecules inhaled through the nose (like cocaine) can also first go to the blood and then cross over the BBB to the brain. Tracking the distribution with real-time imaging has been a challenge, however, because a big halo at the nose obscures the particles' trajectories.

The observed time course suggests that the particles do not travel by diffusion or active transport but by some unknown mechanism. Electron microscopy shows a lack of tight junctions between olfactory sensory neurons, suggesting particles might travel within bundles of axons through the cribiform plate and into the olfactory bulb. Within these bundles of axons, there are channels with evidence of ciliary movement. Once in the brain, a bulk flow mechanism, that is, motion created within the perivascular space by the pulsatile motion of blood flow through the brain, could explain the movement of particles so quickly.

Transient BBB opening

Proteins on the meningococcus bacterium interact with beta-2 adrenergic receptors to open the paracellular route, thus allowing *Neisseria meningitidis* to invade the meninges. Xavier Nassif and his colleagues are working to use this mechanism for paracellular transport across the BBB (Coureuil et al,

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2010). The mechanism leads to a transient opening of the BBB by making the tight junctions between cells temporarily leaky.

Targeting a drug to intracellular destinations in the brain would require an additional mechanism. Alternatively, the technique could be applied at a chosen spot in the BBB close to the intended destination. Disrupting the BBB with a pathogen would be a relatively invasive technique, having significant safety hurdles and implications. However, the use of bacterial fimbriae may serve the purpose in the absence of liver organisms.

Drug Delivery Vehicles

Adeno-associated virus

Viral vectors, especially those derived from adeno-associated virus (AAV) and herpes simplex virus (HSV), allow transgenes to access the intracellular machinery of transcription and translation. Although several virus types are known to cross the BBB, such as HIV and rabies virus, HSV and adenovirus require the targeting of a transcytosis mechanism for delivery across the BBB. AAV can cross the BBB of mice inefficiently; however, thus far, none of the AAV serotypes have been shown to traverse the BBB of primates to any useful extent.

AAV can deliver *HTT* siRNA to brain (Xia et al., 2004; Boudreau et al., 2009). Primate data on the persistence of AAV-delivered transgenes suggest that a single injection provides 10 years of expression, and AAV delivery has been extended to human trials, for example, of Parkinson's disease.

In a single injection to the striatum, AAV infects neurons at very high multiplicities, spreading to several million striatal neurons. In the absence of cell division, AAV fails to integrate into the host chromosome. AAV2 is the most widely used AAV serotype, but AAV5 or AAV9 may actually be better suited for delivery to brain neurons (Foust et al., 2009; Gray et al., 2011).

More research is needed to determine whether AAV-mediated gene therapy could be administered systemically. Systemic delivery would increase the chance of immune effects, which are less of a concern for local delivery. Up to 85% of the population has circulating antibodies to AAV2, which could compromise even initial systemic delivery.

Besides possible immunologic complications, dosing viral gene delivery will depend on the needed multiplicity of infection, available viral titers, and the strength of the promoter used. So far, virally delivered transgenes are constitutively active, so clinical studies would require careful examination of possible side effects and the development of an "exit strategy" in the event that something goes wrong. One possibility would be to use regulatable promoters, though in the past, the FDA has rejected this option out of concern that introducing extra proteins into the construct could itself compromise safety. Another issue, as in the case of direct delivery, is how to achieve a specified level of *HTT* knock-down that is not itself toxic.

Herpes simplex virus

HSV-derived vectors could provide either direct or systemic delivery. Joseph Glorioso has reported that HSV does not naturally cross the BBB, but such ability might be engineered by replacing its machinery for infecting cells with single-chain antibodies that bind to transcytosing receptors, such as transferrin. The modified virus could then be endocytosed into endothelial cells of the BBB and exocytosed to the brain's extracellular space. The choice of an appropriate transcytosing target, however, is challenging since the presence of the target's natural ligand (transferrin) would compete with viral uptake. Other options include the incorporation of other transcytosing viral glycoproteins into the HSV envelope in order to mediate delivery to the brain across the BBB. Such vectors would require detargeting the natural viral receptors to prevent infection of endothelial cells. The detargeted vectors could be supplied with new binding ligands (retargeting) that mediate viral infection of specific neuronal subpopulations in the brain.

HSV is a human virus that infects neurons efficiently and persistently, and it could allow the delivery of multiple therapeutic genes. HSV can express genes long term in neurons, and it can accommodate a DNA cargo up to 40 kb long. The virus does not integrate in the host genome but persists as an extra-chromosomal element in the nucleus. In its engineered vector form, its lytic functions are removed, and it expresses only the engineered gene.

HSV-mediated gene delivery is already in the clinic, with a Phase 2 trial for cancer pain and an early trial for brain cancer (Glorioso and Fink, 2009). In the pain trial, the treatment achieves efficacy with the delivery of a total of 10^8 virus particles. Dosing for HD would likely be different, probably requiring higher doses to breach the BBB. Once established in neurons, however, the vector is highly stable, and repeat dosing might not be needed.

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So far, neither animal experiments nor clinical trials have raised issues with immunogenicity. Most people carry antibodies for HSV, just as they do for AAV, but the dosing level used to date (10^7 to 10^9 /ml) in patients has not proven immunogenic. Nonetheless, long-term dosing will raise safety issues, including immunogenicity, long-term regulation of gene expression, and potential toxicity.

Nanoparticles

Nanoparticles have been used extensively for drug delivery, with some such therapies approved for cancer treatment. Nanoparticles have many functional groups and can conjugate many different molecules. Miqin Zhang's group is developing a 40-60 nm particle with an iron-oxide core coated with a natural polymer called chitosan, present in the exoskeleton of crustaceans (Veiseh et al., 2011). Chitosan is a transcytosing molecule that is able to cross the BBB, and the iron oxide allows the particle to be imaged. The particles are injected systemically and can cross the BBB and deliver drugs to tumors in the brain, with 6-8% of the molecules taken up by the brain. Synthetic nanoparticles might also deliver a gene or siRNA, so it would be possible to try to use the particle to silence *HTT*.

For antisense oligonucleotides and siRNAs, however, the question remains of how to move the nanoparticle cargo into the cytoplasm. One possibility is to use a cell-penetrating peptide, though that would mean limiting the amount of cargo. Another possibility is to make use of existing transporters, for example, the dopamine transporter, but some such strategies may be confounded by HD-associated decreases in transporter concentration.

An endogenous nanoparticle: high-density lipoprotein

Endogenous mechanisms can also carry molecules across the BBB. One such system depends on ApoA1, the major protein component of high-density lipoprotein (HDL). "Nascent HDL" is an ApoA1 molecule surrounding a phospholipid core ~12 nm in diameter. ApoA1 normally acts as a cholesterol acceptor, penetrating tissues and removing cholesterol from fats. In plasma, HDL already carries microRNA, so getting its core protein to carry siRNA might not require major feats of engineering. Also, the structure of ApoA1 is well known, so it's possible to modify it with a single-chain or monoclonal antibody to target it to specific cell types.

ApoA1 performs complex tasks difficult to recapitulate in a synthetic nanoparticle: produced in the liver, ApoA1 travels around the body, picks up its payload, and brings it back to the liver, taking on a variety of structures during its life cycle (Fan et al., 2009). Michael Oda's group has modified ApoA1's structure to deliver both large and small cargos through the pulmonary and transnasal pathways (Oda et al., 2006; Burgess et al., 2010). Some 90% of the cholesterol in circulation exchanges with molecules in the plasma, limiting the utility of this system for reliably delivering cargo. But a more stable form of HDL, further modified by adding polyethylene glycol (PEG), can deliver the highly toxic antifungal compound amphotericin B.

As in the case of synthetic nanoparticles, HDL-derived nanoparticles must not only traverse the BBB and get into brain cells, but also escape the endosomal compartment and deliver the ASO or siRNA to the cytoplasm. AlCana Technologies (Vancouver, British Columbia, Canada) has developed ApoE-dependent systems containing ionizable cationic lipids to deliver nucleic acids to specific cells. The flux of such complexes across the BBB is limiting, so it becomes important to choose especially potent molecules; small molecules, for example, may not be delivered in sufficient quantity to reach an effective concentration. Higher probability of success may require high-capacity transporters and receptors, to minimize potential interaction with the transport of endogenous substrates. Despite the complexity of these systems, the fact that components are naturally occurring reduces safety concerns.

Issues in Translation

CSF and drug delivery

Understanding the flow of CSF is important for predicting the distribution of therapeutic molecules in the brain. This is especially true for drugs delivered by direct administration to the CSF, either intrathecally or intracerebrovascularly. It is also important for identifying biomarkers via CSF sampling and predicting how a drug, once in the CSF, is cleared.

According to the textbooks, cells of the choroid plexus, which line the brain vesicles, secrete CSF into the vesicles; CSF then flows unidirectionally from the ventricles to the cisterna magna, with an unidentified quantity flowing down the spinal column. According to Marijan Klarica, however, this view is incorrect (Vladić et al., 2009; Bulat and Klarica, 2011). Instead, there is no net formation of CSF within the brain

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ventricles; rather, an exchange takes place between CSF and interstitial fluid. Altering the position of the cranium does not change the volume of intracranial fluid, and pressure in the cisterna magna (in head-up position) is normally about zero. Because the volume of the cranium is fixed, the enlargement of the large intracranial blood vessels during systole forces CSF from the ventricles and cortical subarachnoid space into the subarachnoid space of the spinal cord. During diastole, the flow is reversed, so there is continual craniospinal mixing of ventricular, cisternal, and spinal CSF.

Large macromolecules such as proteins are removed from the CSF quite slowly, so they distribute throughout the CSF over time. Klarica's work on distribution dynamics suggests that the concentration of such long-residence-time molecules after 24 h is highest in the lumbar region. Sampling by lumbar puncture should therefore provide a surprisingly good representation of the contents of the CSF. The active mixing of CSF also provides a rationale for intrathecal delivery of brain-directed drugs, as in the case of Isis Pharmaceuticals' ASOs.

Immunogenicity and hypersensitivity

One major concern when taking molecules from the preclinical to the clinical stage is the possibility that they can provoke an innate or acquired immune response in humans. The FDA will probably ask for extensive data to show that a molecule is not immunogenic—not just in rodents, but also in large animals such as primates or dogs.

Immune responses do occur, a concern in all the modalities discussed. A possible exception is the use of nanoparticles to deliver small molecules, though some nanoparticles do cause hypersensitivity reactions in some human research participants. One way researchers have tried to control immune responses to nanoparticles is to treat research participants with steroids and antihistamines. Other approaches are to induce immune tolerance to the carrier particle in advance of treatment and to exclude potentially hyperresponsive patients in advance. No one yet knows whether any of these approaches will eliminate the problem: it may be necessary to accept that 10% of people will not be able to receive a second dose.

Pharmacokinetics and pharmacodynamics

Many factors contribute to whether a drug and its delivery vehicle will perform well. Because the design process contains so much trial and error, some CHDI Foundation Workshop participants have suggested that characterizing a molecule's distribution should await a demonstration of some efficacy in an animal model within a reasonable therapeutic safety window. The importance of measuring drug levels (pharmacokinetics) and the engagement of the potential drug with its presumed target (pharmacodynamics) should be underscored. Without such information, no one would be able to say why a particular molecule might or might not have worked.

Overall Strategy

A drug that shows disease modification in any neurodegenerative disease would help the field, blazing the trail for others. Some have suggested a stepwise approach: start with naked siRNA delivered through a pump to see if it reduces levels of mutant *HTT* in the brain; then find a readout that indicates a desirable change; next deliver the same molecule with a viral vector; and, if successful, move to systemic delivery, perhaps in a viral vector or nanoparticle.

There is general agreement with this staged approach and with the idea that direct intracranial delivery of *HTT*-silencing siRNA offers the current best therapeutic potential, but opinions diverge on the best way to move forward. Alex Kiselyov (CHDI, Los Angeles CA) has suggested the possibility of coadministering an agent with a treatment that opens the BBB. Overall, the global strategy should be using everything that is approved for chronic use in humans.

Pieter Gaillard has noted the parallel between the development of neuroprotective therapeutics and the more mature indication of lysosomal storage diseases (LSDs) (Van Weperen and Gaillard, 2010). To date, the only true disease-modifying approach for LSDs has been obtained in patients using intrathecal infusion (as well as direct intraventricular administration, which is more invasive) of the therapeutic enzymes, and with BBB-penetrant small molecules (substrate reduction therapies). All other approaches (e.g., local or global gene delivery; functionalizing enzymes to target the brain, either specifically targeted or generally by cell-penetrating peptides; nanoparticles; and liposomes) have thus far failed to change clinical practice. Comparing lessons learned there could provide guidance for the HD field.

Many knowledge gaps stand in the way of designing an effective BBB-crossing delivery vehicle and therapeutic for HD and other neurodegenerative

disorders. Do the properties of the BBB change with disease onset and severity? Does brain metabolism, required to clear the nontherapeutic components from the body, differ in individuals with the disease? What brain region does the disease affect first? Should a treatment aim to reverse or arrest the disease after symptoms have already appeared, or is it better to treat before the disease has manifested? Will treating one area of the brain be enough to achieve a therapeutic effect? An additional and crucial issue is the current dearth of biomarkers, both of disease progression and of the engagement of HD-relevant targets.

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