Transporting Therapeutics: A Novel Approach to Deliver ASOs to the Brain

Antisense oligonucleotide (ASO) therapies, which bind to RNA and modify protein expression, are promising drugs for treating neurological conditions. However, their inability to cross the blood-brain barrier (BBB) sometimes makes injection directly into the cerebral spinal fluid (via intrathecal injection) necessary, which is not without its risks or drawbacks (1). However, a recent study by Denali Therapeutics, as well as other scientific research in the field, offers promising techniques to overcome this limitation.

Antisense Oligonucleotides and the Blood-Brain Barrier

The blood-brain barrier acts as a guard to the CNS, barring entry to around 98% of molecules. While this stingy access is essential in maintaining the brain's microenvironment and protecting it from harm, it also excludes helpful drugs, like ASOs, from reaching the brain.

The basic biophysical properties (e.g. size, charge, and backbone chemistry) of oligonucleotides cause them to be quickly cleared from circulation and unable to efficiently cross the BBB (1). Repeated intrathecal or intracerebroventricular dosing directly into the cerebrospinal fluid (CSF) is required to overcome this limitation and access the CNS (1). However, intrathecal oligonucleotide delivery results in reduced drug concentrations in deeper brain regions and increased concentrations in the lumbar spinal cord, which could cause safety issues (1).

Additionally, it comes with the risk of adverse events related to intrathecal dosing that can result in dose limitations or study discontinuation (1). The delivery of "naked ASOs

and siRNAs to most peripheral nonhepatic or renal tissues, such as muscle, are similarly limited because of inefficient functional uptake by muscle cells" (1).

Compared to intrathecal infusion directly into the CNS, BBB crossing molecules — delivered intravenously or subcutaneously — offer a less invasive approach and evenly distribute the drug throughout the brain in animal studies.

Oligonucleotide transport vehicle shown to successfully cross the BBB

Denali Therapeutics recently published research in Science Translational Medicine, that demonstrated that an engineered human transferrin receptor 1 (TfR1) binding molecule acting as an oligonucleotide transport vehicle (OTV) could be used to deliver therapeutic molecules across the BBB to the mouse and nonhuman primate brain (1).

To test the approach with ASOs, scientists focused on a gene called *Malat1*, expressed in all central nervous system (CNS) cell types. The ASO targeting *Malat1* was bound to the transport vehicle and carried into diverse cells (1). Following IV administration, the results showed that the OTV could successfully cross the BBB and produce a cumulative and sustained knockdown of the ASO target gene expression across multiple CNS regions and all major cell types, including endothelial cells, neurons, astrocytes, microglia, and oligodendrocytes (1). Additionally, the OTV enabled knockdown in mouse quadriceps and cardiac muscles, which are historically difficult to target via oligonucleotides (1).

"Not only could we get brain uptake, but we could get it into the cell, we could get knockdown of the genes that we're interested in… Really demonstrating that this platform can be effective for this class of drugs as well," said Denali Chief Scientific Officer Joe Lewcock, Ph.D. in a recent article.

The study also directly compared OTVs with two alternative delivery techniques currently being pursued in the field: ASO conjugated to a high-affinity, bivalent anti-hTfR1 antibody and naked ASO administered intrathecally.

"We demonstrate that OTV resulted in more widespread ASO deposition throughout the CNS compared with both alternative therapeutic strategies. Whereas high-affinity, bivalent antibodies also engage hTfR1 along the brain's vasculature, they cannot effectively cross the BBB into the brain parenchyma, highlighting the unique transcytosis properties of the TV," the study authors explain (1).

The study does have some limitations and states that future studies are essential to better understand the implications of altering dose concentration and frequency (1). Additionally, the authors acknowledge that more work is needed to understand the degree of OTV modularity relating to protein architecture, ASO sequences, chemical modifications, and other ASO RNA targets. Further, because ASO toxicity profiles are sequence-dependent, future OTV studies with therapeutic oligonucleotides need to examine the safety of deposited ASOs in peripheral clearance organs, like the liver and kidney, and the safety of the larger OTV complex (1). Lastly, the authors suggest that further studies examine strategies to improve CNS targeting (1).

Transferrin Receptor and targeting TfR for BBB delivery

The TV technology is based on engineered Fc domains – the tail region of an antibody – that bind to specific natural transport receptors like the transferrin receptor, which is expressed at the BBB and can ferry the TV and its therapeutic luggage to the brain via transcytosis, the brain's active transport system.

To create the vehicle, Denali researchers used combinatorial protein engineering to build a library containing millions of protein variants, each with a slightly different patch of amino acids in the Fc domain. The libraries were then scanned to find variants that could bind to the transferrin receptor, which usually shuttles iron across the BBB. The scanning process, called directed evolution, was repeated several times to engineer specific properties, like improved binding affinity for the receptor. The transport vehicle was then tethered to a therapeutic molecule and tested using an antibody targeting β-secretase 1, an enzyme associated with amyloid build-up in the brains of those with Alzheimer's disease, which effectively crossed the BBB to lower levels of amyloid in the brains of both mice and monkeys.

Denali's CEO, Ryan Watts, explains that while specificity is needed to ensure efficient CNS targeting, it must also be sufficiently active to deliver an efficient dose. Additionally, there must be no potential for Fc-mediated immune responses like antibody-dependent cellular cytotoxicity caused by antibody fusing. A decade ago, Watts and colleagues reported that bispecific antibodies targeting TfR1 and β-secretase 1 (BACE1) – an Alzheimer's disease target – caused low counts of immature red blood cells and signs of hemolysis in mice (2). However, antibodies engineered to be immunologically silent did not cause the same problem. How strongly the antibody fragments bind the receptor also affects their suitability, and Watts and colleagues reported that antibodies with relatively low TfR1 affinity have a better CNS uptake as a high-affinity TfR1-directed antibody may attach too firmly to brain endothelial cells and fail to undergo transcytosis (2).

Denali is far from the only biotech company researching how the transferrin receptor can be used to carry drugs across the BBB. Using its antibody engineering platform, Roche has created antibodies that can cross the BBB by binding to the transferrin receptors located on the endothelial cells lining the blood-brain vessels. Branded as the “brainshuttle,” the technology could also be used to deliver large molecules other than antibodies to the brain (3).

Research into single-domain antibodies, specifically nanobodies, as a potential vehicle for crossing the BBB, is also promising. A nanobody targeting mouse transferrin receptor was fused to neurotensin, demonstrating BBB-transport by receptor-mediated transcytosis (4). Beneficial properties of nanobodies include a high affinity, specificity, and biodistribution, as well as an ability to be engineered and tailored into various formats that broaden their uses (4). For example, the biotech company Ablynx — now part of Sanofi — created the nanobody technology platform. The platform uses

nanobodies derived from llamas, alpacas, and other species whose antibodies contain only heavy-chain peptides (VHH) — in contrast, human antibodies have both light and heavy chains of amino acids. These heavy-chain nanobody molecules are around a tenth the size of conventional antibodies, allowing them to cross the BBB through multiple routes (5).

Peptides, which offer the benefits of being easy to obtain and characterize, are also potential transporters. UK company Bicycle Therapeutics has developed a drug delivery system that uses TfR1 to shuttle RNA therapeutics across the BBB (2). True to its name, the company uses a short synthetic peptide with a bicyclic structure constrained around a central chemical scaffold that binds to TfR1. Bicycle Therapeutics has entered an agreement with Ionis Pharmaceuticals, which has licensed rights to TfR1 binders that deliver oligonucleotide payloads to TfR1-expressing tissues, including muscle and brain tissue (2).

Beyond targeting the TfR1 receptor to cross the BBB, Dyne Therapeutics FORCE platform was also designed to overcome the limitations of oligonucleotide delivery (6). The platform consists of Dyne's proprietary antigen-binding fragments (Fabs), designed to bind to the TfR1 receptor highly expressed on muscle cells, which allows targeted delivery to skeletal, cardiac, and smooth muscle. The anti-TfR1 Fab was conjugated to an ASOs with gapmer chemistry via a cleavable valine-citrulline linker. The therapeutic payload could also be an ASO, phosphorodiamidate morpholino oligomers (PMO), siRNA, or small molecule. The company explains that the platform allows targeted delivery to muscle tissues, extended time between doses, the ability to re-dose, and the ability to target the genetic basis of the disease Myotonic dystrophy type 1 to stop or reverse its progression.

Clinical trials of therapies crossing the BBB

Denali Therapeutics is also using Tfr for their lead drug candidate DNL310, where a recombinant iduronate 2-sulfatase (IDS) enzyme is bound to an enzyme transport vehicle (ETV) and shuttled into cells to treat Hunter syndrome, a disease caused by a mutation in the gene coding for the iduronate 2-sulfatase enzyme.

Currently in its phase 3 trial, DNL310 has demonstrated success in penetrating the brain from circulation. Thirteen patients with Hunter syndrome in a phase 1/2 trial achieved an average 64% reduction from baseline concentrations in neurofilament light chain — a marker of neuronal damage —after two years of the experimental therapy (2). The drug is lauded as one of the most advanced therapies using the iron carrier protein transferrin to cross the BBB. Denali plans to pursue an accelerated approval for DNL130.

The company is also pursuing two OTV candidates for Alzheimer's and Parkinson's disease. However, Denali is not the only pharmaceutical company attempting to solve the problem of transporting therapies across the BBB.

Japanese company JCR Pharmaceuticals is exploring a similar approach to Denail for treating Hunter syndrome and has received approval for its agent, Izcargo, in Japan (2). Izcargo is an anti-TfR1 bivalent antibody that delivers IDS into the brain by TfR1-driven receptor-mediated transcytosis (2). The company has reported successful CNS penetration with Izcargo, improving or maintaining neurocognitive status in 21 of 25 patients in its phase 2/3 trial (2).

The future of ASO drug delivery technology

While there is currently only one FDA-approved gene therapy that can successfully cross the BBB — Zolgensma, a drug used for spinal muscular atrophy in infants — approaches using methods other than TfR to deliver drugs across the BBB are being researched. Voyager Therapeutics, for example, is creating adeno-associated virus (AAV) capsids aimed at crossing the BBB, which its CEO and president, Al Sandrock, believes will be more efficient than AAV9, the naturally occurring AAV serotype. The company discovered that AAV capsids — the outer viral protein shells enclosing genetic material — can be altered on the surface to more efficiently cross the BBB, improving safety and efficacy. Additionally, novel work into using receptors to internalize ligands, allowing the ligand to be transported across the BBB, is also being studied (7). As many receptors (including the insulin, folate, lipoprotein, leptin, diphtheria-toxin, nicotinic-acetylcholine, integrin, scavenger, and interleukin receptors) can facilitate the transport of ligand-conjugated nanocarriers through the BBB, a broad range of ligand-conjugated receptor-mediated drug delivery systems have been explored to treat various brain disorders (7).

Discovering a way to deliver drugs across the BBB could not only improve the safety of oligonucleotide therapies but also improve treatment outcomes. Platforms using the transferrin receptor, like Roche’s brainshuttle, Dyne Therapeutics FORCE platform, VHH nanobodies, Denali’s transport vehicle, and Bicycle Therapeutics, as well as approaches beyond using TfR, all show promise. As all these scientific endeavors continue, a less invasive and more effective future of ASO drug delivery seems likely.

Sources

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