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REVIEW ARTICLE

CNS Targeted Gene Therapies

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ABSTRACT: The rapid advances in the field of gene therapy in the past decade have facilitated the rapid development of many treatments for previously untreatable neurological diseases. Gene therapy creates a long-term treatment for these diseases in comparison to other traditional medications. In recent years, numerous neurodegenerative diseases such as Parkinson's Disease and Alzheimer's Disease have been targets of gene therapy. Alongside the improved efficacy of gene therapy treatments, advancements in efficient vectors of delivery have proved instrumental to the field. This review provides an overview of gene therapy vectors and their delivery methods to the Central Nervous System (CNS). Additionally, this review rationalizes the necessity for further research into promising vectors, such as exosomes for CNS-targeted drugs. Finally, this review highlights the recently developed technology of ultrasound-mediated blood-brain barrier (BBB) disruption which has proven itself to be a promising alternative to traditionally developed CNS targeting mechanisms.

KEYWORDS: Translational Medical Sciences, Disease Treatment and Therapies, Gene Therapy, Central Nervous System, Delivery Vectors.

Introduction

Neurodegenerative Diseases:

Neurodegenerative disease refers to a wide range of highly impactful conditions that relate to neuronal damage in the CNS. The National Institute of Neurological Disorders and Stroke (NINDS) officially recognizes 217 major neurological diseases.[1] These deadly chronological diseases are highly impactful on a large amount of the population. A study by the World Health Organization in 2021 reported that over 3 billion people worldwide were significantly affected by neurological conditions.[2] CNS-targeted gene therapies have grown as a viable long-term treatment option in comparison to short-term traditional medication. Due to the global prevalence of neurological conditions, treatment of such diseases remains an important field of exploration.

The Blood Brain Barrier:

The Blood Brain Barrier (BBB) is a semipermeable epithelium barrier formed by glial cells and brain capillaries. Its function is to act as a barrier between the CNS and outside blood flow. Layered in the glial cell formation of the epithelium are several different transport elements. These transport elements include surface receptors and ion channel protein gates used to facilitate the movement of larger molecules into the brain (Figure 1A). Notably, the iron binding transferrin (TfR) receptor has been a target of many CNS drugs for uptake into the brain. Anatomically, the BBB forms around the blood vessels that circulate the brain (Figure 1B). Due to the tight junctions in the BBB only lipophilic small molecules with a weight of < 400 Da can cross it through passive transport.[3] For this reason, any drug targeted to the brain must be developed with BBB permeation in mind, creating severe limitations on cargo size and delivery vector. To combat this issue, many different mechanisms have been designed to allow for more successful delivery of drug payload.

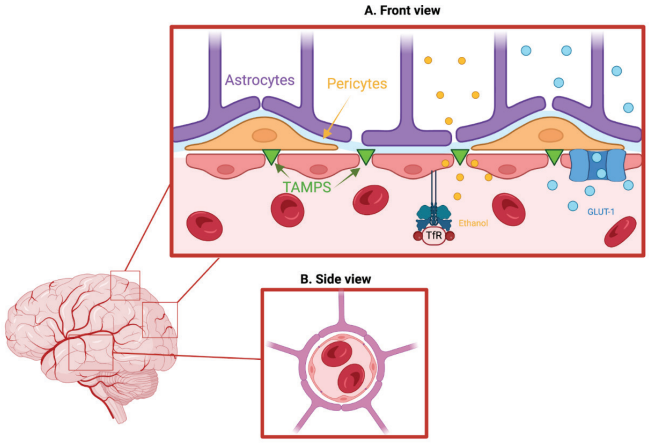

Figure 1: The Blood-brain barrier (BBB) is a selectively permeable membrane enclosing the brain from outside blood flow. Many elements play a role in maintaining its structure: astrocytes repair the BBB, tight junction-associated marvel proteins (TAMPS) maintain the tight junctions and pericytes regulate capillary flow. While some small hydrophobic molecules, such as ethanol, can diffuse across the BBB others require facilitated mechanisms of transport. Surface-attached receptors such as Transferrin (iron and glucose transportation) mediate bulk transport via their binding region. Channel proteins such as GLUT-1 (glucose channel protein) allow for facilitated diffusion of small molecules. BBB composition varies slightly based on the location of the brain, however, all major features are present in each anatomical position. All of these features make the BBB a challenge for any drug delivered to the CNS.

Discussion

Delivery Mechanisms:

Throughout the years, improved targeting mechanisms have greatly increased transduction rates of gene therapies, lowering dosage and increasing drug efficacy. When considering viral vector design, serotype (virus strain) is an important factor. Different serotypes produce varying viral capsids, targeting different types of cells (Figure 5E). The largest obstacle for any CNS-targeting drug is the BBB. Although the BBB is de-

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signed to ensure the safety of the brain, it also interferes greatly with CNS-targeted drug delivery. In the past, most therapies relied on the passive transcytosis conducted by Adeno-Associated Viruses, severely limiting the range of vectors that could be employed. In the past decades, many groups have successfully developed mechanisms to target BBB surface receptors, allowing for higher delivery rates. Some of the most common receptors are the iron-transporting TfR receptor (Figure 1A), the lipid-transporting low-density lipoprotein receptor (LRPR), and the insulin receptors.[4] Currently, 4 main types of delivery mechanisms exist: cell-penetrating peptides (CPPs), Monoclonal Antibodies (MAbs), and surface receptor-targeting ligands (Figure 5). In addition to these traditional targeting mechanisms, ultrasound technology has been explored in recent years as a more economical alternative of CNS targeted drug delivery.

Drug Administration:

To avoid the BBB, different sites of injection have been explored. Currently, the two main methods of drug administration are systematic and direct administration.[5] Systematic delivery involves administration either intranasally or orally. Direct administration involves drug delivery into a vein directly connected to the brain. A method of systematic delivery, the intranasal route has been tested to great success in its ability for BBB avoidance. For example, Efavirenz, a CNS targeting LNP developed to treat HIV, has seen great success with this method of administration. The group developing the drug cited increased CNS targeting rates of 150 times, and absorption rates 70 times compared to that of the orally administered dosage.[5] The biggest limitation of the intranasal route is the severely limited dosage. A method of direct delivery, the intracranial route has been explored as an alternative. The advantage of this method is the proximity to the site of delivery, allowing higher transduction rates. A major disadvantage of the method is the necessity for surgical procedures for injection, causing substantial tissue damage after repeated dosage. In addition to invasiveness, the majority of drug payload is dispersed in the cerebral spinal column, with lower counts reaching the brain.[6]

Vectors Overview:

- ***Viral Vectors:***

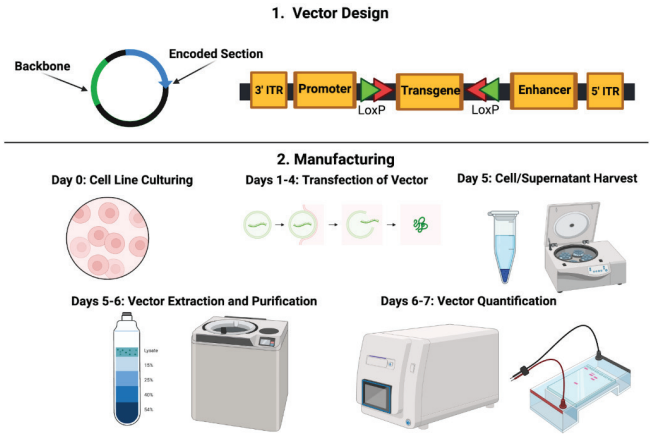

Figure 2: Viral vectors are composed of two inverted terminal repeat (ITR) sections enclosing the coded section of the vector. The encoded section includes the promoter, LoxP sites (Optional), the transgene, and enhancer segments. During vector production, several steps are followed to ensure the highest purity of the extracted vector. Vector transfection time is highly dependent on the specific vector. All of these steps are critical for efficient and effective vector usage.

A viral vector is a form of virus, whose genome has been edited to only contain necessary components for its use in gene transfer. Viruses undergo DNA/RNA synthesis in two processes: the lytic cycle and the lysogenic cycle. The greatest difference between the two is the lysogenic cycle involves gene integration of the virus into the host genome through transposons. In comparison, the lytic cycle only conducts transcription through its circular episomal DNA inside the nucleus, independent of host chromosomes. In its recombinant form, viral vectors have their unnecessary genetic backbone cut out during production. The typical vector is composed of two inverted terminal repeat sites (ITR) at the 3' and 5' ends flanking the coded section of the gene (Figure 2). Inside the coded section of the gene first comes the promoter, which regulates gene expression and cell specificity. Afterward, the gene of choice (transgene) is inserted. Sometimes vectors employ the loxP system, which consists of two 34 bp palindromic sequences flanking the transgene. During translation the loxP system utilizes Cre recombinase to achieve knock-in of the transgene, effectively sealing it in place. Depending on the size constraints of the vector, sometimes enhancer genes are utilized to improve vector efficacy.[7] An example of this is the commonly employed PolyA gene which codes for a PolyA tail, preventing enzymatic degradation of the vector.

The general workflow of vector production on the scale is to first culture the desired cell line, then transduce the vector into the culture, and finally harvest the completed vectors after purification[8] (Figure 2). Further details about viral vector development can be found in this protocol for AAV development.[9] Some of the main advantages of viral vectors over non-viral vectors are long-term transgene expression, high transduction rates into target cells, and simple methods of production.[10] The largest drawbacks of viral vectors are their limited transgene size, cytotoxicity, immunogenicity, and re-

duced scalability compared to non-viral vectors.[10] Another important consideration when employing all types of viral vectors is the immunological response against the introduction of the treatment. To counteract this immunological response, many groups have found success in utilizing immunosuppression before treatment.[11] In this review, the most common CNS-targeted viral vectors will be reviewed; these include Adenoviruses (ADVs), Adeno-Associated Viruses (AAVs), Retroviruses, Herpes Simplex Viruses (HSVs), and Lentiviruses (specific family of retroviruses).

- ***Non-Viral Vectors:***

A non-viral vector is an organic chemical compound engineered to effectively deliver genetic material to its target. The typical composition of a viral vector mimics that of a phospholipid bilayer, with the cargo inserted in the inner compartment (Figure 2A). The advantages of non-viral vectors in comparison to viral vectors are their increased scalability, lower cytotoxicity, elimination of gene integration risk, and greater flexibility in genetic cargo size. The largest drawbacks of non-viral vectors are their lower gene expression, more complex methods of production, and lower transfection rates compared to viral vectors.[10] One of the major issues of all non-viral vectors for CNS delivery is their large molecular size, which requires them to employ various mechanisms for BBB transcytosis. In recent years, non-viral vectors have seen greater usage. For example, the Lipid Nanoparticle (LNP) vector used in the Pfizer COVID-19 vaccine has displayed the potential of nonviral vectors in mRNA delivery.[12] The most common CNS-targeted non-viral vectors that will be reviewed include LNPs and exosomes.

Viral Vectors:

- ***Adenoviruses:***

Adenovirus (ADV) is a medium-size (90-100 nm) non-enveloped icosahedral virus that contains double-stranded DNA (dsDNA).[13] In their recombinant form, ADVs can accommodate up to 36 kilobase pairs (kbps), making them a great choice for larger transgenes.[13] The three greatest advantages of the ADV vector are its efficient large-scale production, well-defined biology, and high transduction efficacy.[14] In terms of gene expression, ADV vectors are only able to undergo the lytic cycle and remain in episomal form upon transduction into the host cell.[13] Although there were many concerns regarding the safety of ADV as a vector of delivery, safe practices have been well-established since its creation.[13] ADV has remained one of the most researched vectors, alongside its counterpart the AAV. The high immunogenic response generated against ADV has severely limited its usage as a CNS targeting vector.[15] Some groups such as Castro *et al*. have seen clinical success in implementing ADV vectors for specific use cases. This treatment, done for Glioblastoma Multiforme utilizes the high transduction rates, safety profile, and high levels of transgene expression of ADV.[16] Specifically, the treatment introduces cytotoxic genes that allow for tumor suppression by mediating tumor cell death. Nevertheless, ADV remains one of the less common CNS targeting vectors currently being employed.

- ***Adeno-Associated Viruses:***

Adeno-associated virus (AAV) is a small-size (25 nm) non-enveloped icosahedral virus that contains single-stranded DNA (ssDNA).[17] In its recombinant form, ADV can accommodate approximately 4.7 kbps.[18] Due to the small packaging size of the vector, efficient packaging of the intended transgene is crucial to AAV design. Since their discovery as an ADV containment in 1965, AAVs have been developed to be the main CNS targeting vector.[19] The three main advantages of AAV vectors are high stability, efficient crossing of the cellular membrane, and low host integration risk.[20] In terms of gene expression, AAV vectors mostly conduct the lytic cycle, although they rarely can integrate at site-specific locations on the host genome.[20] Due to the minimal immunological response elicited by AAV vectors, they have shown great promise in gene therapy overall. Accordingly, the first two gene therapies to ever receive FDA approval used AAV vectors of serotypes 2 and 9.[21] For CNS-targeted drugs, many AAV vectors have been developed for many different diseases. For example, AAV-GAD, a vector targeted for Parkinson's disease has been approved for FDA fast-track development.[22] The drug is currently being tested in phase two and has shown promising results. Overall, AAVs are the most common CNS targeting vector currently being employed, which can be attributed to their small molecular size. In some cases, AAVs can passively permeate through the BBB without any targeting mechanisms.

- ***Retroviruses:***

Retrovirus is a large-size (80-100 nm) enveloped non-icosahedral virus that contains ssRNA.[23] In its recombinant form, retroviruses can accommodate approximately 10kbps.[23] The greatest advantage of Retroviruses is that they can conduct both the lytic and lysogenic cycles. This allows for greater long-term gene expression and reduces the necessity for frequent dosage. Retrovirus utilizes reverse transcriptase to convert its RNA into DNA and inserts its DNA into the host genome through transposons.[24] The concern of laboratory cross-contamination remains a key issue for the employment of retrovirus.[24] Nevertheless, in the 30 years of clinical applicability, no major issues of retroviral malignancies have been noted. One of the major disadvantages of the Retrovirus is that it only targets dividing cells. Because the majority of neuronal cells remain in G0 for their lifetime, they are non-dividing. Consequently, retroviral vectors are unable to effectively target many neuronal cells. Retroviruses have however seen great success in exo vivo transduction treatments.[25] Due to their high transduction efficacy, retroviruses hold great promise for autologous/allogeneic vaccines which involve modified patient-derived cells.

- ***Herpes Simplex Viruses:***

Herpes simplex virus (HSV) is a large-size (125 nm) enveloped round icosahedral virus that contains dsDNA.[24] In its recombinant form, HSV can accommodate up to 150 kbps, one of the largest packaging sizes of any vector.[24] This allows for the packaging of multiple, large transgenes. In terms of gene expression, HSV only undergoes the lytic cycle, remain-

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ing in episomal form. HSV was first noted as a possible vector of gene delivery when scientists discovered long-lasting latent HSV in the peripheral nervous system.[25] This key trait of wild-type HSV virus targeting neuronal cells makes it a potential vector of delivery for CNS-targeting drugs. Currently, HSV is being investigated by several groups in the clinical stages of CNS disease treatment. For example, HSV-1716 - which was developed by Sorrento Therapeutics uses a HSV vector for Malignant Pleural Mesothelioma. In their phase 1/2a study half of the enrolled patients observed disease stabilization.[26] Overall, HSV has great potential as a CNS targeting vector for high-carrying size drugs.

- ***Lentiviruses:***

Lentiviruses are a subclass of mammalian retroviruses that are known for their slow incubation period. Human Immunodeficiency virus (HIV) is the most common mammalian variant developed for vector transduction. In its natural form, Lentivirus is a large size (80-120 nm) enveloped round icosahedral virus that contains ssRNA.[27] As a subclass of Retrovirus, Lentiviruses also contain reverse transcriptase and undergo the lysogenic cycle. The main advantage of Lentiviruses in comparison to retroviruses is their ability to target both dividing and non-dividing cells. This allows Lentiviruses to effectively target non-dividing neuron cells. In its recombinant form, Lentiviral vectors (LVs) can accommodate 9.2kbps.[27] Lentivirus's ability to target both dividing and nondividing cells has made them a great option for STEM cell delivery.[28] A study done in 2007 looked into the viability of LV as a CNS-targeted vector by utilizing a targeting mechanism.[29] The group induced a recombinant protein on the vector surface targeted towards the low-density lipoprotein receptor (LDLR). LDLR is an abundant receptor on the BBB surface, allowing for the uptake of lipoproteins into the brain. After analyzing the transduction efficacy of the GFP transgene which was encoded into the vector, the study cites an uptake efficacy of astrocytes of 84% across the entire CNS.[29] Lentiviruses show great promise as both an *in vivo* and ex vivo vector of delivery.

Non-Viral Vectors:

- ***Lipid Nanoparticles:***

Lipid Nanoparticles (LNPs) are a class of nonorganic vectors primarily used for mRNA delivery. Their sizes range from 50 nm to 200 nm, the relative size constraining the cargo limit. In terms of gene expression, LNPs do not pose any risk of gene integration. Pfizer's LNP mRNA COVID-19 vaccine has brought greater attention to the potential of LNPs as an RNA delivery vector. The structure of an LNP is composed of a structure that mimics a phospholipid bilayer. The propulsion between the hydrophobic heads of the inner compartment (s) and the outer compartment creates the space for the cargo (Figure 3). The four main components of an LNP are ionizable cationic lipids, DSPC, cholesterol, and PEG-lipids. Ionizable cationic lipids serve the function by allowing the encapsulation of nucleic acid contents and disrupting the outer membrane to allow for the escape of contents into the cytosol upon delivery. DSPC improves endocytosis and cellular delivery of the vector.[30] Cholesterol is dispersed throughout the outer membrane of the vector to increase the half-life and decrease the amount of surface-bound proteins.[31] PEG-lipids are the main structural scaffold of the LNP and mainly make up the inner and outer membranes. The length and density of PEG are critical to avoid the accelerated blood clearance(ABC) phenomenon, which occurs in high-dose injections.[31] LNPs can carry various types of genetic cargo, including DNA, siRNA, microRNA, and mRNA.[32] Currently, LNPs are being explored for the treatment of a multitude of different diseases such as amyotrophic lateral sclerosis, Parkinson's disease, and Alzheimer's disease.

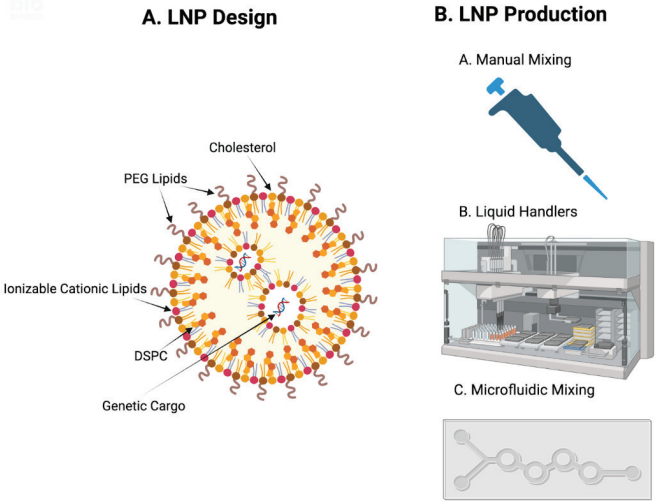

Figure 3: All LNPs have four main components: Ionizable cationic lipids, DSPC, Cholesterol, and PEG-lipids. Each plays a critical role in maintaining the structure and fluidity of the vector. There exist three main methods of LNP production: manual mixing, liquid handling, and microfluidic devices.[33] Manual mixing is the simplest method, however, it creates more imbalance in molecular mass distribution. Liquid handling has the greatest automation, however, the size created cannot be regulated as well. Microfluidic mixing devices are generally recognized as the most reliable method, however desired size is not guaranteed. LNP design has been modified in many cases to create a vector suited to the need.

- ***Liposome/Exosomes:***

Exosomes are a class of naturally occurring cell-derived vesicles, ranging from 30 nm to 130 nm.[34] In a similar construct, the liposome is composed of a lipid bilayer without the protein components contained on exosome surfaces. Due to the natural process of derivation, exosomes have increased immune tolerance when deployed. For this reason, they are excellent for fields of immunotherapy such as Ex-vaccination, a method of transporting viral vectors to bolster the immune system.[34] Endosomes secreted from Dendritic Cells (DCs) and B cells are enriched with MHC1, MHC2, and costimulatory (such as B7/CD80) molecules are then loaded up with the tumor antigen for T cell presentation. The subsequent simulation between the T cell receptor and the antigen presented on the surface, alongside the costimulatory B7 and CD28,[34] activates the T cells. Currently, there are few CNS treatments utilizing exosome vectors undergoing clinical trials. Recently, AB126 which was developed by Aruna Bio was approved for clinical trials 1a/2b due to their unique BBB crossing vector.[35] This vector holds great promise for modulating inflammatory reactions in

the CNS, especially for certain diseases such as Amyotrophic Lateral Sclerosis.

Vector	Carrying Capacity	Size	Integration	Cytotoxicity	Gene type
ADV	36 Kbp	90-100 nm	Episomal	High	ssDNA
AAV	4.7 Kbp	25 nm	Episomal, Chromosomal	Low	ssDNA
Retrovirus	10 Kbp	80-100 nm	Episomal, Chromosomal	Low	ssRNA
HSV	150 Kbp	125 nm	Episomal	High	dsDNA
Lentivirus	9.2 Kbp	80-120 nm	Chromosomal, Episomal	Low	ssRNA
LNP	Variable	20-200 nm	Episomal	Medium	Variable
Exosome	Variable	30-130 nm	Episomal	Low	Variable

Figure 4: This table illustrates five key characteristics of each vector described: Carrying capacity, size, integration, cytotoxicity, and DNA type.[13] Retroviruses and Lentiviruses contain reverse transcriptase and thus are able to achieve gene integration readily. Adeno-associated viruses do not contain reverse transcriptase; however, they are able to integrate into the chromosome at specific locations. Each viral vector contains either double-stranded(ds) or single-stranded(ss) RNA/DNA. Non-viral vectors are able to encompass any of these types of genes.

Delivery Mechanisms:

- ***Ligand Conjugation:***

Vector surface ligand conjugation has been established as a popular method for conducting receptor-mediated transcytosis on the BBB surface (Figure 5b). In the example of the transferrin receptor (TfR), a ligand is conjugated to exhibit a high TfR binding domain. In the past, a major limiting factor for LNPs for CNS-targeted treatments was their inability to cross the BBB. However, many companies such as DNLI therapeutics have developed mechanisms to counteract this issue. DNLI has created a unique transport vehicle (TV) for small-scale molecular drugs such as LNPs that target the TfR receptor.[36] The TV utilizes a TfR high affinity binding domain on a surface attached ligand to allow attached cargo to enter the CNS. An entire class of LNP's known as 'Molecular Trojan Horse LNP's' have been created using this concept.

- ***Monoclonal Antibodies:***

A monoclonal antibody (mAB) is a laboratory-made antibody modified for a specific purpose. The most common subtype of antibody in use utilizes the IgG (subclasses 1,2, and 4) domain.[37] In most cases, only certain regions of the antibody are utilized, and fused with the desired vector for maximum efficacy. In the case of CNS targeting drugs, the purpose of the mAB is to enable receptor-mediated endocytosis across the BBB. The variable regions of the antibody heads are modified for binding to the corresponding receptor. This method has applicability to all vectors, as binding of the mAB to the vector surface can be achieved. A study done in 2017, targeted the TfR receptor that is highly expressed on BBB surface cells. The group utilized a liposome vector (140 nm) with a surface-attached mAB (named OX26) conjugated for high-affinity TfR binding.[37]

- ***Cell Penetrating Peptides:***

A cell-penetrating peptide (CPP) is composed of a few short (15-25) links of amino acids containing many positively charged amino acids.[38] Due to the negatively charged nature of cellular membranes, the positively charged ends of the CPP can effectively penetrate through the membrane. In particular, to CNS targeting drugs, CPPs have been explored as a mechanism of BBB permeation by a couple of groups to conflicting results. A study done in 2015 looked into CPP efficacy for BBB permeation and the possible immunological effects.[39] The study concluded that CPPs selectively permeate the BBB and could not prove any change compared to passive diffusion. However, another study in 2022 utilized a different variant of CPP with greater results. The study reported close to 100% transduction, however, only to peripheral endothelial cells.[40] Further research is required to validate the results proposed by both studies and if for specific cell populations CPP's could remain a possible field of exploration.

- ***Ultrasound:***

Ultrasound-mediated BBB disruption has been explored in recent years as a cost-effective alternative to targeting mechanisms. The two ultrasound devices used for therapeutic applications are single-element and multi-element devices. In general, multi-element ultrasound devices hold more promise due to their greater spatial coverage.[41] The premise of the technology is to utilize highly concentrated ultrasound waves to temporarily disrupt the BBB and allow for drug delivery during that short period.[41] There have been several different approaches explored for the applicability of this technology. For example, a study in 2022 utilized ultrasound to penetrate the BBB and allow for the delivery of a liposome vector.[41] The majority of vectors currently being tested with ultrasound technology are some form of lipid-encoated bubble. Despite the obvious economic applications of this technology, there remain some concerns regarding cytotoxicity. In higher dosages, the temporary disabling of the BBB has been shown to cause higher counts of damage associated with molecular patterns (DAMPS).[42] Subsequently, these DAMPS have been shown to elicit an autoinflammatory response from the innate immune system, creating an inflammatory response. Overall, BBB-targeted ultrasound is a promising new field of exploration for CNS treatment that requires further development.

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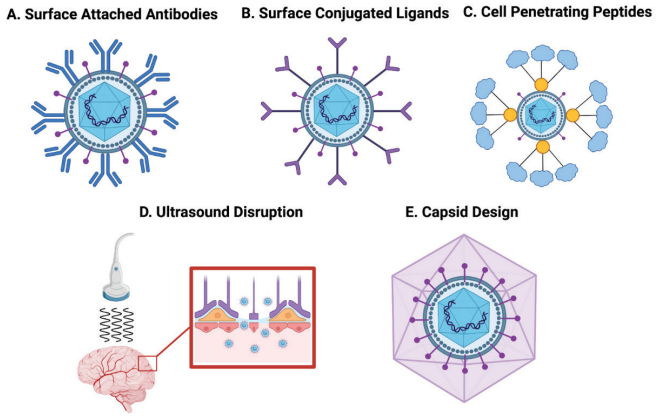

Figure 5: Targeting Mechanisms
Targeting mechanisms are critical to ensure proper targeting of the vector. **A.** Surface attached antibodies: The variable region of the antibody is modified to enable receptor receptor-mediated transcytosis (RMT) of the attached vector. **B.** Surface conjugated ligands: A guiding ligand is attached to the vector surface and conjugated to induce RMT. **C.** Cell-penetrating peptide (tricyclic): Three short links of amino acids are attached to the vector surface, allowing for penetration of the Blood Brain Barrier (BBB). **D.** Ultrasound disruption: Ultrasound waves are amplified and targeted to specific locations to temporarily disrupt the BBB, allowing for drug delivery. **E.** Capsid design: Efficient vector capsid involves optimization of the capsid programming region of the vector and the serotype variant chosen.

Conclusion

This review discussed six different vectors currently being employed in CNS-targeted gene therapy: Adenoviruses, Adeno-associated viruses, Retroviruses, Herpes Simplex Viruses, Lentiviruses, Lipid Nanoparticles, and exosomes. A comparative table of the different properties of each vector can be found above (Figure 4). Due to the wide variety of vectors currently employed for different circumstances, it is crucial to understand the advantages and drawbacks of each vector for efficient drug development. In particular, exosome vectors hold great promise as CNS targeting vectors due to their low cytotoxicity and high carrying size. Further research is necessary to determine the possibility of exosome usage as a CNS-targeting drug. Currently, AAVs remain the most commonly employed viral vector for CNS targeting due to their low molecular size and high transduction rates. LNPs are the most common non-viral vector due to their low cytotoxicity and high production rates.

In recent years, ultrasound technology has been developed as a promising alternative to traditional vectors. Due to its unique mechanisms of targeted BBB disruption, drugs delivered through ultrasound do not need to contend with the restrictions placed by the BBB. In addition, the procedure is non-invasive and low-cost. While ultrasound faces some safety issues due to the temporary BBB disruption it causes, it has been proven to be mostly safe. This method would allow for greater streamlined development of CNS targeting drugs, especially geared towards rarer genetic diseases. Currently, many companies are undergoing clinical trials for ultrasound-mediated BBB disruption.

Acknowledgments

Figures: All Figures created with BioRender.com
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