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Review of Existing Method for Brain Delivery

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Abstract. Central nervous system (CNS) diseases represent a growing global health concern, driving growing attention of researchers towards developing effective brain delivery methods that can cross the brain-blood barrier (BBB). This paper reviews the recent advances in the BBB-penetrating strategies for treating CNS diseases with detailed description and comparative analysis of various BBB-penetrating techniques, including both non-invasive and invasive methods. Non-invasive method include intranasal injection, intracerebroventricular injection, focused ultrasound, neurotropic virus-mediated delivery, and methods employing cell transcytosis - subdivided into carrier-mediated transcytosis (CMT), receptor-mediated transcytosis (RMT), adsorption-mediated transcytosis (AMT), and paracellular pathway. Notably, hybrid nano-particle systems are widely used in non-invasive methods. Invasive techniques, such as intrathecal delivery, intracerebral injection, and intra-arterial injection are also discussed. While non-invasive methods, particularly RMT, AMT, and focused ultrasound, are more effective brain delivery tools for their safety and target efficiency, invasive injection methods can achieve higher drug delivery but are constrained by the localized distribution of drugs on the BBB. Despite the many improvements in BBB penetration strategies, challenges including boosting selectivity and reducing cytotoxicity continue to require research and attention.

Keywords: Blood-brain Barrier, Drug Delivery Strategies, CNS Diseases.

1. Introduction

Central Nervous System (CNS) diseases are one of the leading causes of death and disability [1]. With over 3 billion people suffering from neurological conditions, CNS diseases pose a heavy burden on our society [1]. Throughout the years of research, several key challenges have emerged: the complexity of understanding CNS diseases, the limited objective measures of disease severity and progression, the difficulty in patient identification, and the lack of efficient technologies for the delivery of drugs across

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the BBB. In this review, one of the significant challenges is discussed and addressed, the delivery of therapeutic agents into the brain, namely, across the blood-brain barrier (BBB).

Among these challenges, crossing the BBB poses a critical obstacle when treating CNS diseases, considering it hinders the development of new drugs, thus limiting the treatment options for CNS diseases. Though the BBB is recognized for its prominent role in maintaining the brain, it substantially limits the ability of many medications intended for the CNS. As it is often assumed, large molecules would be blocked by the CNS, and small molecules are able to freely diffuse through the barrier. However, It is estimated that over 98% of potential small-molecule neurotherapeutic agents are blocked by these barriers from reaching the CNS [2]. Therefore, a significant blockade of treating CNS diseases is crossing the BBB.

Recent advances in drug delivery took the approach of initiating a BBB disruption or opening the BBB directly to enter the BBB. Methods aligning with these principles include utilizing ultrasound to open the blood-brain barrier [3], binding to receptors on the cells that line the vessel walls [4], and antibodies that inhibit a receptor responsible for maintaining the integrity of the blood-brain barrier [5]. There has also been growing interest in increasing BBB permeability through technologies primarily designed to create brain lesions, such as low-intensity focused ultrasound (LIFU) [6] and laser interstitial thermal therapy (LITT) [7]. These advances emphasize the need to reconsider some concepts on brain drug delivery and also reveal a great need to discuss the current strategies, and by building on them, develop new approaches in crossing the BBB.

In this review, we discuss the recent advances in our understanding of the BBB, focusing on the physiological characteristics that impede the drug molecules crossing the BBB. We then review the various approaches and strategies to deliver drug molecules across the BBB with their side-effects discussed, while focusing on new approaches developed and investigated in the past 3 years. Notably, strategies are divided into two sections: the noninvasive strategies and the invasive strategies. Finally, a summary of the findings and future directions for further studies will be provided.

2. General Background on the Blood-Brain Barrier

The blood-brain barrier (BBB) is a selective semi-permeable membrane between the blood and cerebral tissue of the brain. It primarily took the role of maintaining CNS homeostasis by protecting the brain from the extracellular environment [8]. Notably, the blood-brain barrier acts not only as a protective shield but also as a selective gateway for substances to enter and leave the brain, a producer of certain compounds, and a site for local chemical reactions involving enzymes. [9].

Anatomically, the BBB includes the following components: the tightly packed endothelial cells (ECs), pericytes, the capillary basement membrane, tight junctions (TJs), and astrocytes [8]. EC cells, which line all blood vessels, are commonly known to regulate the exchanges between the bloodstream and the surrounding tissues [10]. In the BBB, ECs are polarized along two axes of a blood vessel into two types: luminal (blood-facing) and abluminal (brain-facing) plasma membrane domains [11]. Pericytes are cells found along the walls of capillaries. They are primarily associated with the stabilization and hemodynamic processes of blood vessels [12]. The last three components are unique to the BBB and will be elaborated in the following sections. While structurally, the blood-brain barrier is also a part of the neurovascular unit (NVU), which together regulates the matching of blood flow to local neuronal activity, thereby neurovascular coupling [13].

The loss of these unique characteristics to the BBB is, in fact, a key factor in the pathophysiological characteristics of neurological conditions like stroke, multiple sclerosis (MS), brain injuries, and neurodegenerative diseases [14]. Moreover, invasive methods that open the BBB are often connected with BBB disruption, which can result in ion dysregulation, edema, and neuroinflammation, eventually leading to neuronal dysfunction, increased intracranial pressure, and neuronal degeneration [9].

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2.1. Healthy BBB condition

In the healthy state of BBB, as previously mentioned, it acts as a highly selective barrier that prevents the entry of toxic chemicals and brings in the essential nutrients. This is also where all of the components of BBB, namely, the ECs, pericytes, TJs, and astrocytes fulfill their roles [8].

The endothelial cells (ECs), being at the core of the BBB, differ in function from the rest of the body. Unlike most endothelial cells that keep blood vessels relaxed and open enough to let through blood, ECs on BBB exhibit no fenestrations, extensive tight junctions (TJs), a high abundance of efflux transporters, and sparse pinocytic vesicular transport [8]. EC tight junctions limit the paracellular flux of hydrophilic molecules across the BBB and thus provide high resistance to paracellular transport [15]. Efflux transporters, such as the ABC efflux transporter P-glycoprotein (P-GP), actively pump a wide range of lipophilic drugs out of the brain ECs that form the BBB, thus serving as another defense against toxins [16]. Additionally, the low level of pinocytic vesicular transport poses yet another barrier to transcellular passage [17].

Pericytes are cells embedded in capillaries and postcapillary venules that wrap around the endothelial cells. They regulate the structural integrity and differentiation of microvessels, and the formation of endothelial TJs [18]. Outside the endothelial cells form a boundary that separates the ECs from the pericytes, and astrocyte end-feet - the capillary basement membrane (BM). It acts as a filtration barrier and regulates cell proliferation and differentiation [19].

Aside from the structural components aforementioned, another key feature of the BBB is the organization of the multiprotein junctional complexes, including the adherens junctions, which regulate endothelial barrier permeability; TJs, which maintain the integrity of the epithelial and endothelial barriers; and gap junctions, which are composed of connexin, facilitate direct communication between endothelial cells (transmitting both electrical and chemical signals) [8]. Together, they aid the BBB in regulating CNS homeostasis.

2.2. Pathological BBB condition

The integrity of the blood-brain barrier can be compromised in various health issues, including stroke, diabetes, seizures, conditions like hypertensive encephalopathy, AIDS, head trauma, multiple sclerosis, Parkinson's, and Alzheimer's disease.

Following stroke reperfusion, for example, the cerebral vasculature experiences a reduction in integrin expression and the degradation of the surrounding extracellular matrix, leading to an increase in leakage of the BBB [20]. For diabetes, *Liu H et al.* have found that streptozotocin-induced diabetes mice exhibit BBB disruption with altered P-glycoprotein (P-GP), where the level of P-GP in the brains of the diabetic rats examined was significantly lower than that of control rats [21]. While in neurodegenerative diseases like Alzheimer's disease, *Take Y. et al*, have demonstrated that the exposure of EC cells to Aβ leads to actin organization disruption and death of ECs in vitro, again allowing more Aβ and other neurotoxic substances to enter the brain, exacerbating neuronal damage [22].

The disruption of the BBB in these pathological conditions often arises from several shared mechanisms. One of the key factors in BBB disruption is the uncontrolled inflammation following injury or disease. In such cases, there will be an elevation of pro-inflammatory cytokines, which weakens BBB's integrity [23]. Mitochondrial dysfunction resulting from diseases may elevate oxidative stress, inducing lysosomal dysfunction and the autophagy of key components of the BBB [24]. Usually, following injury and in pathological disease states, the matrix metalloproteinases (MMPs) are upregulated for purposes such as tissue remodeling and more [25]. However, they are found to degrade the TJ proteins and basal lamina proteins, leading to BBB leakage [26]. Together, these processes interconnect, reinforce one another, and eventually lead to a progressive breakdown of the BBB.

3. CNS Drug Molecules

For a drug molecule to bypass the BBB, it must fulfill the following physical and chemical properties, such as size, hydrophilicity, and polarity. Past studies have demonstrated that the ideal characteristics

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of a CNS drug molecule exhibit the following characteristics: a molecular weight of less than 500 g/mol, lipid solubility, electrical neutrality, and a weak base [27].

However, despite the careful designing of drug molecules, certain shortcomings in their current formulation call for the need for change. For instance, in treating Alzheimer's, aducanumab, a monoclonal antibody that targets amyloid-beta plaques has the issue of passing through BBB without causing BBB disruption [28]. Similarly, monoclonal antibodies, such as those used in cancer treatment (e.g., trastuzumab), are typically too large to cross the BBB [29], limiting their effectiveness in treating brain tumors.

Drug molecules primarily follow one of the 3 mechanisms to open the BBB: (1) increasing BBB permeability (via the administration of inflammatory substances or utilizing methods opening the BBB directly); (2) bypassing it via direct brain delivery (including intracerebral delivery and intrathecal delivery); and (3) modifying the therapeutic agents to utilize natural transport mechanisms (such as transcytosis and the lipophilic pathway). For example, one popular approach was chemically modifying the therapeutic agents or loading them in BBB-permeable carriers [30]. Other strategies involve using targeted pharmaceutical agents [31] or high-osmolarity infusions [32] to temporarily open the BBB.

4. Noninvasive Delivery Methods

Methods used to carry drugs across the BBB can be divided into non-invasive methods and invasive methods. This section introduces main non-invasive injection across the BBB, including intranasal injection, intracerebroventricular injection. Focused ultrasound is discussed as a non-invasive external BBB crossing method. Additionally, non-invasive methods also makes the use of endogenous transcytosis pathway such as carrier-mediated transcytosis, receptor-mediated transcytosis, adsorption-mediated transcytosis, paracellular pathway, and neurotropic virus-mediated blood-brain barrier delivery.

4.1. Intranasal Injection

The intranasal route, which is a type of transmucosal delivery, offers an effective and noninvasive way to get drug molecules into the brain. Herein, intranasal refers to the administration of drugs through the nasal cavity. The transport mechanism involves two different pathways, such as olfactory pathway and trigeminal nerve pathway [33]. These two pathways initiate in the brain and terminate in the nasal cavity at the olfactory neuroepithelium or respiratory epithelium. In the case for both systems, drug molecules bypass the BBB to enter the CNS [33].

The key to the mechanism of intranasal delivery of drug molecules is the diffusion of therapeutic agents across the nasal mucosa in the olfactory region of nasal cavity which has direct access to the CNS [34]. The journey of these molecules from the nasal area to the brain's tissue is made possible via pathways that follow either the olfactory nerves, which are related to smell, or the trigeminal nerves, which are connected to facial sensations. By directly injecting pharmaceuticals into the nose via local spray administration (by an atomizer that sprays the surface of the nasal mucosa), the drug molecules would enter the nasal cavity, and move along the olfactory or trigeminal nerves, finally arriving at the parenchyma of the brain [35]. Upon reaching the starting points of the nerves in the cerebrum and pons, the molecules then spread throughout the brain using two different routes: one that travels within the cells (intracellular) and another that moves outside the cells (extracellular). [33].

4.1.1. Intracellular Pathway

In the intracellular mechanism, pinocytosis would occur, internalizing the molecule by an olfactory neuron. Following internalization, the cargo-containing endosome is trafficked down the axon, thus performing neuronal translocation [33]. Once the endosomes reach the CNS, drug molecules are exocytosed and released into the postsynaptic cells of the olfactory bulb [33].

However, the process of neuronal transport is regarded as a rather slow process. In mice, the olfactory nerve is approximately 4nm in length and about 20mm within the trigeminal [36]. This means that the vesicles have to cover this intracellular distance to reach the CNS. *Crowe et al.* estimated the time for

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an intranasally administered drug to reach the brain for the olfactory nerve axonal transport alone, and concluded with a 45 min time period; however, other processes such as diffusion within the nasal cavity, endocytosis, trafficking, and exocytosis are not included in these calculations and thus prolong the time in practice [33]. On the other hand, *Crowe et al.* have also estimated that, when all of these processes are included, it would take between 0.74 and 2.67 hours for a substance to be transported the length of the olfactory neuron. For the trigeminal, this range would be approximately 3.69 and 13.33 hours [33]. For velocity values of transport, *Buchner K et al* have conducted an ex vivo study, in which they estimated two values: a slow rate with 36 mm per day, and a fast transport mechanism with 130 mm per day [37].

4.1.2. Extracellular Pathway

The extracellular pathway starts with the drug crossing the nasal epithelium to the lamina propria driven by the paracellular movement between olfactory neurons and supporting cells, then it would be transported externally via axonal transport toward the olfactory bulb [33].The drug can move from the lamina propria, which is a layer of tissue, into the space surrounding the olfactory neurons. This space is between cells that wrap around the olfactory neurons and other cells called olfactory neural fibroblasts (ONFs). This movement happens by a process called diffusion. [33].It's worth noting that the movement of substances through the space around the nerves, known as the perineural space, happens much quicker than when they're transported inside the cells along the nerve fibers.

Lochhead J et al. calculated the time needed for passive diffusion on the length of the olfactory and trigeminal nerves. Based on their previous experiments and information from other studies, they figured out that it takes about 0.73 to 2.3 hours for a substance to move from the nose's surface layer (nasal epithelium) to the olfactory bulb [36], which is a part of the brain involved in smell. They also guessed that it would take somewhere between 17 to 56 hours for the same substance to travel from the nose's surface layer to another part of the brain called the pons, but this time following the trigeminal nerve. Thus indicating a more efficient and feasible route of the olfactory route compared to the trigeminal route.

4.1.3. Drug Molecules Examples

Research indicates that the nasal administration route is appropriate and effective for delivering a wide array of substances, including both influenza and polio viruses, as well as molecules of varying sizes such as aluminum lactate (with a molecular weight of 294 Daltons), insulin (with a molecular weight of 5.8 kiloDaltons), and even gold particles (measuring 50 nanometers in size).[33]. It is particularly suitable for drugs with lower molecular weight and higher lipophilicity, and the drug metabolism in the nasal cavity, degree of dissociation (pKa), chemical structure, drug half-life (t½), osmolarity and pH should also be considered [38].

Popular drugs for nasal delivery, such as antihistamines and corticosteroids, are commonly used to suppress histamine-induced wheal responses and flare reactions in allergic rhinitis (Table 1). These drug molecules are known to treat nasal discomforts and were developed around 2010, giving itself a relatively long history. But more recently, *Borrajo, M. L. et al.* have found that mRNA vaccines, which encode viral proteins to stimulate an immune response, may also be delivered through the intranasal pathway, giving itself a list of advantages such as needleless, stimulating systemic immunity, but also providing protection at the site of infection [43]. In this study, nanoparticles (NE) are used to carry these vaccines, containing either ionizable lipid (C12-200) or cationic lipid (DOTAP), improving cellular uptake and ensure the mRNA reaches the cells where it can effectively trigger an immune response. The intranasal delivery may also be applied to neurodegenerative diseases like Parkinson's. In another study, *Castellani, S. et al.* have carried dopamine (DA) and citicoline (CIT), which are crucial in re-establishing normal brain and cerebral cortex structure, inside solid lipid nanoparticles, providing controlled release and improved bioavailability of these neuroprotective agents [45].

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Table 1. Examples of Drugs and Excipients Used in Intranasal Delivery.

Drug	Excipient	Drug Properties	Condition(s) treated	Note	References
Antihistamines	Fluticasone furoate, etc.	Suppress histamine-induced wheal response and flare response	Allergic Rhinitis & Vasomotor Rhinitis	Fluticasone furoate is used for its high affinity to glucocorticoid receptors, providing anti-inflammatory effects	[39]
Corticosteroids	Lactose	Reduce the production of chemicals that cause inflammation	Allergic Rhinitis	Lactose acts as a filler or bulking agent in corticosteroid	[40]
Levocabastine	Benzalkonium chloride, etc.	Antagonize histamine H1-receptor	Relieve allergy symptoms in the nose	Levocabastine is a classic antihistamine formulated for rapid relief of allergic symptoms.	[41]
Temozolomide (chemotherapeutic)	Chitosan lipid nanoparticles	Prevents DNA repair in cancer cells	Glioblastoma	Chitosan lipid nanoparticles enhance drug delivery across the blood-brain barrier and improve stability.	[42]
mRNA vaccines	NE with an ionizable lipid (C12-200) NE with cationic lipid (DOTAP)	Encodes viral proteins to stimulate immune response	Various infectious diseases	NE (nanoparticles) with an ionizable lipid (C12-200) and cationic lipid (DOTAP) improve cellular uptake and endosomal escape.	[43]

4.1.4. Advantages and Limitations

The intranasal route offers distinguishing advantages such as the ease of administration, rapid onset of action, and avoidance of first-pass metabolism [47]. Moreover, it is less painful than intravenous or intramuscular administration, needle-free, quick to deliver, and suitable for both children and the elderly [35]. However, despite its advantages, intranasal drug delivery suffers from low permeability of the nasal mucosa to polar molecules, limited residence time (due to rapid mucociliary clearance) and

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presence of metabolizing enzymes [48]. It may also have usage limitations, such as being unsuitable for use in individuals with nasal trauma or septal defects.

4.2. Intracerebroventricular (ICV) Injections

Intracerebroventricular drug administration, a method that bypasses the blood-brain barrier, allows the direct entry of high drug concentrations to the CNS by injecting the drug molecules into the cerebrospinal fluid (CSF) [49]. Perhaps being the least studied among various routes of drug administration, the ICV injection held great potential for the delivery of drug molecules.

The ICV injection begins with the injection of drug molecules into the cerebral ventricles approaching it either by directly injecting drugs into the CSF [50]. Cerebrospinal fluid (CSF) is the fluid that surrounds the brain and the spinal cord that has a buffering and protective effect. Cerebrospinal fluid is produced by the choroid plexus of the lateral ventricles, and when the drug enters the CSF, it passes through the interventricular foramen to the third ventricle, then through the cerebral aqueduct to the fourth ventricle, and finally through the median foramen of the fourth ventricle to the subarachnoid space. Next, the cerebrospinal fluid circulates rostrally to the choroidal absorption site. Notably, this is achieved through the implantation of the ICV device. The implantation involves the surgical insertion of a catheter or cannula into the lateral ventricle of the brain, connected to an external or subcutaneously placed reservoir. The drug molecules are then absorbed into the peripheral bloodstream across the arachnoid villi, which are small protrusions of the arachnoid membrane into the dural sinuses of the brain, to enter the general circulation. Similar to the intravenous pathway, the injected drug molecule enters the CSF and exits, then rapidly enters the peripheral blood, and then re-enters the brain across the BBB to induce its effects [51]

When approaching the ICV route, the following two methods are considered: intralumbar administration and the Ommaya reservoir implantation. However, the intralumbar administrations resulted in adverse reactions that included back and leg pain, difficulty voiding, and temporary paresis [52]. On the other hand, the Ommaya reservoir presents a better alternative. The Ommaya reservoir consists of a mushroom-shaped dome made of thickened silicone rubber catheter enabling needle punctures and is implanted in the ventricles of the brain [52]. The ventricular catheter is inserted into the reservoir capsule, with its distal end positioned in the anterior horn of the ventricle [52]. The proximal end is connected to the reservoir capsule, creating a complete cerebrospinal fluid delivery system [53]. The drug is then injected into the reservoir capsule via a port located on the scalp, allowing for slow and controlled release into the cerebrospinal fluid [53]. From there, the drug diffuses throughout the central nervous system via cerebrospinal fluid circulation.

4.2.1. Drug Molecules Examples

The ICV pathway is a popular administration method for most analgesic and antispasticity drugs with the exception of morphine. This method is also favored for the administration of antibiotics and antineoplastic medications because it facilitates a more uniform distribution of the drugs throughout the cerebrospinal fluid (CSF) space. [52]. In terms of the selection of drugs for this administration method, preservative-free drug solutions are preferred and solution pH, osmolarity also must be considered in order to minimize the risk of chemical arachnoiditis [52]

One established example of this method is vancomycin, a tricyclic glycopeptide antibiotic to treat infections [54]. ICV administration of vancomycin has been used successfully to treat meningitis, ventriculitis, and intracranial device infections with gram-positive organisms [55]. Another example may be aminoglycosides, a class of antibiotics to treat severe infections. Through a study of 6 adolescent and adult patients with gram-negative meningitis, *Kaiser and McGee* found that when the drug is delivered through the intramuscular or intravenous pathway, it shows ineffective CSF concentrations; but when delivered via the ICV route, the drug gives a superior distribution of the drug in the CSF [56].

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4.2.2. Advantages and Limitations

Notably, the ICV administration method offers several benefits, including its effectiveness in delivering drugs directly to the central nervous system (CNS), penetrating the BBB, being a reversible procedure, and its ability to use smaller amounts of medication compared to systemic administration, which greatly reduces the risk of systemic side effects. However, this method may also carry certain limitations. First, it is a more invasive procedure than intrathecal administration, as it requires the placement of a catheter through the brain parenchyma and into the lateral ventricles. It may also lead to various adverse effects, including nausea, drowsiness, somnolence, mental clouding, visual hallucinations, miosis, headache, dizziness, pruritis, diaphoresis, urinary retention, and constipation [57]

4.3. Focused Ultrasound

Focused ultrasound (FUS) is a strategy that employs ultrasound to open and or disrupt the BBB in highly targeted brain areas[58]. It delivers drug molecules via promoting the movement of drugs transported in the bloodstream into the brain [58]. Although the success of FUS as an effective strategy for drug delivery in preclinical trials, the actual mechanisms by which the BBB is disrupted remain unclear. There are multiple hypotheses and or factors proposed that may together contribute to the result of the disruption of the BBB via FUS and each of the following are discussed below.

Microbubble Vibration Mechanism

This hypothesis proposed that the FUS waves cause the microbubbles to vibrate and consequently result in the mechanical activation exerting force on the capillary walls, widening the TJ on the BBB, and thus opening the BBB [59].

Microbubble-Induced Stress Mechanism

Another proposed hypothesis is the stress on vessel walls theory. Two factors play a role in this theory: the stress from the vessel walls and the microstreaming-induced circumferential stress. Through in vitro experiments, Chen H. et al. demonstrated that as the bubbles expand and contract, they put more pressure on the walls of the blood vessels. It was noticed that the blood vessel walls get drawn into the center of the vessel, and this might be what breaks the blood-brain barrier. [60]. In addition, the pressure around the microvessel that leads to the opening of the BBB may also be due to the circular stress caused by the blood flowing around the vibrating microbubble in a pattern known as microstreaming [61]. Together, all these mechanical forces can activate special channels in the cells lining the blood vessels, which might cause the blood-brain barrier to break down [62].

Other Factors

Inertial cavitation is also believed to be another promising factor for BBB opening. The inertial cavitation is connected with high-speed micro-jetting and extreme local temperature rise and these factors are suggested to cause BBB opening [61]. Additionally, studies of the spectral information indicated that harmonics and ultraharmonics correlates with the opening of BBB [63].

4.3.1. FUS delivered Drug Examples

FUS has been applied to deliver numerous therapeutic agents in preclinical trials. For example, for brain tumors, FUS has been used to increase the accumulation of etoposide in mouse brain tumor tissue. In one study, mice were exposed to FUS seven days after tumor implantation, then given etoposide. Results showed that the concentration of etoposide in the sonicated tumor tissue was also over 8-fold higher than in the non-sonicated tumor tissue, implying that FUS aids in the opening of the BBB and increases intratumoral delivery of etoposide in the brain [64]. In a recent study for Alzheimer's disease, a specific anti-Aβ antibody is delivered into a mouse model of AD (5XFAD) by microbubbles-enhanced FUS-mediated BBB disruption. It was found that though antibodies do not normally pass the BBB, it can be delivered across via FUS and this result was consistently reproduced in ten mice [65]. Similarly, another study combined FUS with intravenous neurotrophic (protein or gene) delivery. *Karakatsani M et al.* found that this combination of treatment attenuates the damage to the nigrostriatal dopaminergic pathway and more importantly, can slow the rapid progression of Parkinson's disease while improving

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the neuronal function in its early stages [66]. However, due to multiple reasons, including the vague understanding of the mechanism of FUS, FUS is not yet applied to clinical uses.

4.3.2. Advantages and Limitations

There are 3 major advantages for the use of FUS as a way for temporary BBB disruption. Firstly, FUS is non-invasive, which not only prevents the risk caused by the surgery or anesthetic, but also enhances the capability for multi-times treatments for future use. Secondly, introducing MRI, an imaging method for targeting the brain, enables the FUS to be led to the target brain areas. As an example, this would be advantageous for tumors where there is limited access to the chemotherapeutic treatment of brain tumors. Lastly, BBB opening with FUS is transient and closes 6 hours after treatment. Once closed, the BBB can function as usual and remains closed [67]. Previous studies have shown that FUS does not produce long-term deficits in barrier function [68]. However, since FUS works with BBB disruption, there are inevitable side effects. Side effects have to do with either the thalamotomy lesion or perilesional edema and include ataxia, gait disturbance, dysgeusia, paresthesias, and hemiparesis [69]. Additionally, *Paff M. et al.* have conducted a study in search of sensory side effects, where they found 17 out of 107 patients to develop sensory side effects after thalamotomy persisting 3 months after the procedures [69].

4.3.3. Recent Development

In recent years, SDT (Sonodynamic Therapy) with MRgFUS (magnetic resonance-guided focused ultrasound) and magnetic resonance (MR) imaging-guided high intensity focused ultrasound (MRgHIFU) have attracted much attention. SDT employs ultrasound in tandem with a sonosensitizer to specifically target and harm cancer cells. On the other hand, MRgFUS (Magnetic Resonance Guided Focused Ultrasound) utilizes high-intensity ultrasound waves to accurately focus on tumor tissues, and it can also disrupt the blood-brain barrier to improve the delivery of therapeutic drugs. [70]. At the same time, MRgHIFU, similar to MRgFUS but with high intensity, has its unique treatment of musculoskeletal tumors. *Cabras, P et al.* have recently introduced a novel modular MRgHIFU device that enables the precise and interactive positioning of the ultrasound transducer over the targeted body part to be treated [71].

4.4. Carrier-mediated transcytosis

Carrier-mediated transcytosis (CMT) refers to an automatic passive molecule transport route via solute-like transporters (SLC) [72]. In CMT, the molecule combines with a solute transporter and is shuttled across the BBB [73].

CMT is applied to transport only small and hydrophilic molecules [74]. Small and hydrophilic drugs or agents are transported across the BBB via the combination between the drug and the natural ligand of the transporter [74]. For instance, according to the study of Gynther et al., ketoprofen, a drug that is not the ligand of large neutral amino acid transporter 1(LAT1), is conjugated to LAT1 and the result showed that ketoprofen is successfully brought across the BBB [75].

CMT has great biocompatibility for the application of endogenous transporters. The main CMT transporters include carbohydrate transporters, amino acid transporters, the solute carriers (SLC) family, monocarboxylic acid transporters, hormone transporters, organic anion and cation transporters, fatty acids, nucleotide transporters, amine transporter, choline transporter, vitamin transporters [76].

Among carbohydrate transporters, glucose transporters (GLUTs) are one major carrier extensively applied for drug delivery. For example, the research by Qu et al. in 2014 designed glucosides-modified liposomes loaded with docetaxel, which enables the liposome to bind the glucose receptor 1 (GLUT-1) and cross the BBB [77] Also, GLUT are commonly used for brain cancer therapy by limiting the amount of available glucose via the reduction of GLUTs.

As for amino acid transporters, LATs and excitatory amino acid transporters (EAATs) are the major transporters. LAT1 shuttles large neutral amino acids (e.g., L-LEU, L-VAL, L-HIS) and drugs (e.g., levodopa and gabapentin) [78-79]. For example, levodopa, the prodrugs of dopamine, can cross the BBB by LAT1 and transform to dopamine in the brain with the assistance of peripheral inhibitors such

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as aromatic L-amino acid decarboxylase (AADC). Glutamate with excitotoxicity is transported by EAATs as well.

Organic anion transporters (OATs) and organic cation transporters (OCTs) is capable of bringing in non-steroidal anti-inflammatory drugs. OAT3, OATP1A4, and OATP2B1 can transport organic anions such as indoxyl sulfate, benzylpenicillin aminohippuric acid; OCT1, OCT2, OCT3 can transport organic cations such as morphine, MPTP and creatinine [76]. Monocarboxylic acid transporters, in particular MCT1 and MCT2 can move the lactose out of the brain to the bloodstream [76].

Besides, selecting the BBB-penetrating molecules via screening as surrogate ligands of CMT is an effective way to discover new CMT pathways [80].

4.5. Receptor-mediated transcytosis

Receptor-mediated transcytosis (RMT) is a primary transcellular route to shuttle therapeutic macromolecules-receptor complex across the BBB via clathrin-mediated or caveolin-mediated endosome vesicles [81]. RMT is regarded as one of the most promising pathways for therapeutic brain delivery. RMT can deliver transferrin (TF), insulin, lipoproteins, leptins, amyloid beta, tumor necrosis factor alpha (TNFalpha), IGF [82], and other drugs.

4.5.1. Mechanism of RMT

The mechanism of RMT breaks down into three steps: endocytosis, intracellular vesicular delivery and exocytosis [83]. For endocytosis, the drug first binds to the receptor on the luminal side, forming a drug-receptor complex. Then for intracellular vesicular delivery, the complex is internalized and transported across the brain endothelium cells via clathrin-mediated vesicles, caveolin-mediated vesicles, or macropinocytosis vesicles [83]. Finally, for exocytosis, the sorted vesicle releases the drug on the abluminal side and the receptor is recycled [83]. Additionally, drug using RMT system takes the advantage of the endogenous receptor mediated pathway by designing drugs with similar epitope as the ligand of the receptor.

The internalization pathway of intracellular vesicular delivery can be mediated by clathrin or caveolin. Clathrin-mediated endocytosis (CME) pathway is used by nearly 95% of the transmembrane receptors for internalization, in particular TfR and insulin receptor [84]. After internalization, clathrin-coated vesicles (CCV) fuse with early endosomes. The early endosome can either transport cargo across the cells via sorting tubules or mature into the mutil-vesicular bodies where the cargo is degraded by the lysosome [84]. However, cargo entered the lysosome can be retrograded back to Golgi apparatus then to the early endosome. Caveolae-mediated vesicles can directly carry the cargo across the cells without endosome sorting process.

4.5.2. RMT Receptors

In RMT, there are a variety of receptors that play a significant role in brain delivery. To target these receptors, delivered drugs are usually loaded in nanocarriers modified with peptides or the functional part for recognition. In this section, the property and application of commonly used RMT receptors are introduced, including transferrin receptors, lactoferrin receptors, lipoprotein receptor-related protein, insulin receptors, diphtheria toxin receptors, and other receptors (Table 2).

4.5.2.1. Transferrin Receptors (TfRs)

TfRs are membrane glycoprotein that uptake iron from plasma glycoprotein [85]. Transferrin receptors such as human heavy chain ferritin receptors (TfRs) are the most frequently used receptors for brain delivery. To use RMT for drug delivery, studies often add pieces of peptide of HFn onto nanoparticles, enabling the nanocarriers to be recognized by TfRs.

The application of HFn for drug delivery can prevent the drug degradation in peripheral tissues and to increase the selectivity in RMT. For example, in 1996, Jörg Huwyler et al. first used liposome to target the BBB. They sterically stabilized the immunoliposomes with polyethylene glycol (PEG) and grafted the OX26 monoclonal antibodies (mAbs) on the surface to recognize rat transferrin [85].

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Fan et al. proved that human heavy chain ferritin (HFn) could bind to transferrin receptor 1 (TfR1) for sharing the same receptor with transferrin (Tf) with relatively high drug loading/releasing efficiency [86]. After binding to TfR1, HFn accumulates in endosomes, while the drug CSN effectively escape from lysosomes and cross the BBB [86]. Based on this HFn mediated mechanism, Federica Palombarin et al further developed a hybrid nanoparticle to deliver RNA aptamers. They humanized Archaeoglobus fulgidus ferritin (HumFt) and grafted it with the epitope of HFn for TfR1 targeting. Also, a positively charged polyamine dendrimer (PAMAM) cell is assembled inside HumFt, the new hybrid nanoparticle can bring negatively charged nucleic acids across the BBB via TfR1 receptor mediated transcytosis [87]. Also, in the study of Clark et al., the ability of BBB penetration of the gold nanoparticle was increased by establishing an acid-cleavable linkage with the transferrin (Tf), resulting in high selectivity to target TfR1 on endothelial cells [88].

4.5.2.2. Lactoferrin Receptors (LfRs)

Lactoferrin (Lf) is another glycoprotein member of the transferrin family. Ligands of LfRs are highly expressed on the BBB. Therefore, LfRs are commonly used for functionalization in LfR-mediated transcytosis. For example, a Poly (ethylene glycol)-poly (lactide-co-glycolides) (PEG-PLGA) nanoparticle coated with Lf can load shikonin (SKH) and target glioma cells across the BBB via RMT [89][110].

4.5.2.3. Lipoprotein Receptor-Related Protein (LRP)

LRP is a special lactoferrin receptors with multi-ligands. They have multiple ligands such as Amyloid-beta, insulin, IGF-I, leptin, transthyretin, ApoE and etc [90][111]. LRP plays a great role in both clearance from CSF and drug delivery via RMT.

First, lipoprotein receptor-related protein (LRP1) removes Aβ in the brain into the circulation. Aβ first binds to the domain of LRP1 on the abluminal side, forming a PICALM/clathrin-mediated vesicle loaded with Aβ-LRP1 complex, and the PICALM will guide Aβ-LRP1 complex to an early endosome and then the sorting endosome which releases Aβ to the bloodstream in the luminal side [76].

On the other hand, LRP1 can be used for drug delivery. LRP1 is highly expressed in glioblastomas, and Angiopepes-2 is the ligand of LRP1 stemmed from Kunitz domain-derived peptides. It can cross the BBB via LRP mediated transcytosis. Therefore, covalently binding Angiopepes-2, paclitaxel (ANG1005) can be transported across the BBB [91]. However, since Angiopepes-2 is the only useful for ligands in brain delivery via LRP1, artificial peptides L57 (TWPKHFDKHTFYSILKLGKH-OH) is designed to improve the absorption toward drugs across the BBB [92].

4.5.2.4. Insulin Receptors (IRs)

IRs are also highly appeared on the BBB [93]. For example, Karsten Ulbrich et al conjugated human serum albumin (HSA) nanoparticles (NP) with anti-insulin receptor monoclonal antibody (29B4) to enable the penetration of BBB [94].

4.5.2.5. Diphtheria Toxin Receptors (DTRs)

Heparin-binding epidermal growth factor (HB-EGF), also called DTR, is an endocytosis transport receptor in the RMT system. This receptor has several advantages: (1) little competition or blocking toward the DTR-mediated transcytosis for the scarsitiy of DTR ligand in human body; (2) high expression in the brain with disease conditions; (3) easy modulating under a variety of compounds such as heparin. Diphtheria toxin (DT), the ligand of this receptor with well-known basic information such as the receptor binding domain, makes this transcytosis method easy-modulated [95].

Therefore, DTR-mediated transcytosis is considerably promising. For example, by functionalization, DTR is capable of effectively carrying nanogels with deoxyuridine and targeting the glioblastoma cells where the deoxyuridine in the nanogels can be incorporated into the tumor cells' DNA [96].

4.5.2.6. Other RMT Receptors

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Other receptors in RMT include nicotinic acetylcholine receptors (nAChRS), leptin receptors, folate receptors, integrin receptors, scavenger receptors, and interleukin receptors [97].

Table 2. Receptors on BBB used in RMT

Receptor	Functionalization	Nanocarrier	Disease	Reference
Transferrin receptor	Transferrin	Transferrin-coupled liposome	Ischemic ischemic brain injury	[98]
		Tfpep-gold nanoparticles (AuNPs)	Glioma cancer	[99,88]
		Gold nanorods (AuNRs)169	Neurodegenerative diseases	[100]
	Receptor binding domain of HFn	PLGA-NPs	Glioma cancer	[101]
		A.Fulgidus HumFt	Nucleic acid	[102]
	Anti-transferrin receptor antibody (OX26)	Immunoliposomes -PEG	Daunomycin	[83]
		Chitosan-PEG-biotin-avidin/OX26	anticaspase peptide Z-DEVD-FMK	
Insulin & Insulin-like GF receptor	Insulin	AuNPs	Neurodegenerative disease	[104]
	Anti-inuslin receptor antibody (29B4)	Human serum albumin	loperamide	[94]
LRP1 and LRP2	Angiopep-2 & L57-ANG1005	Angiopep-2-ANG1005 complex	Recurrent brain metastases of pretreated breast cancer	[92]
Leptin receptor	leptin-derived peptide (Lep70–89)	polyethylene glycol (PEG)-LPs	CNS Diseases	[105]
Lactoferrin receptor	Lactoferrin	PEG-PLGA NPs	Glioblastomas	[86]
Nicotinic acetylcholine recepotrs	DCDX	liposome	Glioblastomas	[95]
Diphtheria toxin receptor	Receptor-binding domain of diphtheeria toxin	RDT-NP	Glioma cancer	[96]

4.5.3. Advanced method for RMT

Recent studies found that screening ligands for nuclear receptors with RNA sequencing and gene ontology is prospective. For example, to enhance the transportation of leptin through the BBB, Shi et al. analyzed the sequence of 73 nuclear ligands in a library to find the one that can increase the amount of leptin in the brain, and used transcriptome profiling to further test the increase of leptin the screened contributor [106].

4.6. Adsorption-Mediated Transcytosis (AMT)

Adsorption-mediated transcytosis (AMT) is a noninvasive intracellular drug delivery mechanism that utilizes charge interactions between drug molecules and cell membranes to facilitate transmembrane transport of drug molecules.

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The surface of the EC membranes in brain capillaries is covered with a large number of clathrin-coated pits and vesicles, including sialo-glycoconjugates and heparan sulfate proteoglycans [107]. These pits open on the luminal surface of the ECs and carry a negative charge, which repels similarly negatively charged molecules. Therefore, the AMT pathway usually only allows the transport of positively charged drug molecules or carriers, enabling them to bind to the negatively charged regions of the cell membrane through electrostatic attraction.

When a positively charged drug molecule binds to the cell membrane, the cell membrane locally takes it in to form vesicles in a process called endocytosis. During endocytosis, drug molecules are encapsulated in endosomes, where they are carried along the intracellular transport pathways to the opposite side of the cell. After a series of intracellular transport, the cell membrane invaginates again, forming vesicles that expel the drug-containing endosomes, thus releasing the drug via exocytosis. The basal side of ECs, which is adjacent to the basement membrane, also forms a negatively charged barrier due to the presence of glycosaminoglycans such as chondroitin and heparan sulfate-rich GAG components. The consecutive electrostatic barriers formed by the cell surface on the luminal side, the cell surface on the abluminal side, and the basement membrane together create a selective environment for positively charged substances [108], thereby facilitating drug delivery across the BBB into the parenchyma. This method may be applied for the drug delivery and treatment of various CNS diseases. [109].

4.6.1. Modifications for Enhanced AMT

Since the mechanism of action of AMT is delivery through charge interactions between drug molecules and cell membranes, some negatively charged drugs cannot be delivered across the BBB via the AMT pathway. To address this issue, researchers proposed delivering them through positively charged transfer carriers, such as PEG and Polyethylenimine (PEI) [110], or by chemically modifying the drug molecule to exhibit a positive charge, enabling it to bind with the membrane. For example, the simplest approach is to amide the carboxylic acid moiety of a drug molecule (i.e., the carboxy-terminal, glutamic acid (Glu) and aspartic acid (Asp) side-chain moieties) with positively charged amines (e.g., carbodiimide and hexamethylenediamine, etc.) to enable the drug molecule to carry a positive charge [111].

A more studied approach is the use of cell-penetrating peptides (CPPs) coupled with drug molecules to promote the binding of drug molecules to membranes [112]. CPPs are short peptides consisting of less than 30 amino acids. With properties to penetrate cell membranes and amphiphilic and net positively charged sequences, CPPs are capable of carrying proteins, polyskins, nucleic acid fragments and other active substances into cells. Currently, several studies have found that CPPs can not only improve the drug transport efficiency, but also, by selecting different types of CPPs, improve the targeting ability of drug molecules and drug penetration.

In one study, *Zhang et al.* investigated CB5005 CPP and found that it not only improves the transport efficiency of doxorubicin across the BBB, but also enhances drug delivery by targeting and inhibiting nuclear factor kappa-B (NF-κB) activation in gliomas, thus indicating significant anti-tumor effects [113]. *Rusiecka et al.* synthesized a covalent complex of TP10-dopamine, which showed higher therapeutic activity than L-DOPA in an animal model of Parkinson's disease. The dopamine bound to TP10 was able to enter into the brain tissues more readily and showed decreased sensitivity to catechol-O mono-methyltransferase that has a higher affinity for dopamine D1 and dopamine D2 receptors [114]. In addition, CPPs can also be attached to non-natural amino acids (e.g., D-type amino acids, L-4 benzoyl phenylalanine, etc. [115], thus avoiding recognition and degradation by proteases *in vivo*, further enabling CPPs to penetrate the BBB efficiently. The application of CPPs in the mechanism of AMT provides an effective strategy to improve the ability of drug molecules to penetrate the BBB and by designing new CPPs, the AMT pathway may satisfy a variety of drug delivery needs.

4.6.2. Advantages and Challenges of AMT

Additionally, since AMT does not require any specific receptor-mediated drug delivery and heavily relies on physicochemical interactions, i.e., charge interactions, to cross the BBB, the versatility of the

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delivered drugs is significantly increased. In addition, it is less restrictive on the size of drug molecules and can help large molecule drugs to cross the BBB because of its transcytosis nature. The AMT pathway provides a new direction for the treatment of a variety of CNS disorders, which has great potential and application value in the field of intracerebral disease therapy. However, although AMT holds large prospects and significance in the subject of intracerebral disease treatment, there are still several limitations that exist. For example, the relatively low specificity of AMT's binding may lead to potential adverse effects; the possibility of cationic CPPs being captured by negatively charged intracellular structures like lysosomes could reduce the availability of drug molecules outside the cell and affect their bioavailability; the long-term safety of AMT also remains unclear and requires further research [116].

4.7. Paracellular pathway

The paracellular pathway, known as the intercellular pathway, is a route that heavily relies on the tight junctions, adherens junctions, and gap junctions between the endothelial cells of the BBB. These junctions separate the brain parenchyma from the systemic circulation, and thus ensures the integrity of the BBB and consist of a variety of proteins including claudins, occludins, tricellulins, and Zonula Occludens [117]. They bind to the endothelium tightly, forming a channel that allows only specific substances to pass through. It primarily transports small water-soluble molecules and certain ions, while restricting the passage for large drug molecules and lipophilic substances [118]. In this case, the negative concentration gradient from blood to brain mainly drives the drug passage, in which the drug molecules passively diffuses into the brain.

4.7.1. Paracellular Junctions and Transport Mechanism

Compared to the peripheral ECs, brain microvascular ECs are more tightly arranged. Even under severe pathological conditions, the increased permeability of TJ in the BBB renders a particle size of up to 20 nm to cross the BBB. Therefore, one of the research directions is to artificially induce tight junctions' openings to facilitate the entry of the candidate drug molecules into the brain. Recent studies have found that vascular epidermal growth factor (VEGF) and smoothened signaling pathway play pivotal roles in maintaining the stability of tight junction proteins [119]. By interfering with the smoothened signaling pathway or down-regulating VEGF, the tight junction proteins could be destabilized, thus increasing BBB permeability. Another approach is by injecting vasoactive substances such as bradykinin, histamine and their analogs into the bloodstream to open the BBB. These injected substances could transiently increase intracellular Ca2+ concentration by agonizing B2 receptors on ECs, which in turn activates nitric oxide synthase and increases nitric oxide concentration in the bloodstream. This causes vasodilation and opens the tight junctions of the BBB [120]. In addition, opening the BBB can be assisted by physical stimulation and injecting hypertonic solutions (Table 3). Kroll & Neuwelt were the first to open the BBB with hypertonic mannitol in the clinic, which could improve the transport of chemotherapeutic agents [121]. Ting et al. used the technique of focusing and microbubbling in combination with ultrasound to improve the penetration of carmustine across the rat's BBB[122]. Recently, researchers have also utilized RNA interference (RNAi) to target components of the BBB (e.g., cytoplasmic articulin component ZO-1). In this approach, RNAi downregulates different mRNA transcripts through the endogenous degradation system to specifically open the BBB. By downregulating the targeted proteins such as cytoplasmic articulin component ZO-1, RNAi increases BBB permeability [123].

Another research direction to enhance drug molecules' ability to cross the BBB via the paracellular transport pathway is to focus on the drug itself. By utilizing computer-based molecular simulations, the drug's structure can be modified to adjust molecular groups, increase hydrophilicity, reduce molecular weight, and thereby improve penetration capability. Recent research papers have found that phosphatidylinositol-3 kinase (PI3K) inhibitors can promote glioblastoma (GBM) cell death. Through structural optimization, a large number of PI3K inhibitors (such as BKM120, GDC-0941, PX-866, etc.)

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are currently under research, with some already in clinical trials, providing new therapeutic approaches for GBM treatment [124].

Table 3. Methods for Modulating Blood-Brain Barrier Permeability and the effects of their action.

Methodology	Drug Molecule	Effects	Reference
Hypertonic solution	25% mannitol, arabinose, etc.	Increases the osmotic pressure of brain capillaries, shrinks endothelial cells, and opens tight junctions, leading to increased permeability of the blood-brain barrier.	[158]
Vasoactive substances	Histamine, bradykinin, cereport, LipoBridge, etc.	Increases intracellular Ca2+ concentration, induces synthesis of NO, increases NO concentration, dilate blood vessels,open tight junctions.	[120]
Signaling pathway	Smoothened inhibitors, cyclobenzaprine, vascular epidermal growth factor, etc.	Decreases the stability of protein tight junctions, thereby increasing BBB permeability	[119]
Physical stimulation	Focused ultrasound (FUS), electromagnetic fields, microwave radiation, etc.	Transcranial delivery of low-frequency ultrasound is utilized to open tight junctions between BBB endothelial cells	[159]

4.7.2. Advantages and Limitations of the Paracellular Pathway

The paracellular pathway offers a natural route for crossing the BBB, allowing the transport of small molecules and specific ions to diffuse with respect to the concentration gradients without requiring energy. However, this pathway is non-specific and non-selective, which poses limitations and challenges. While drug molecules can cross the BBB, other substances, such as pathogens and neuroinflammatory mediators, may also enter the brain unchecked. This could potentially worsen CNS diseases rather than improving them. This pathway is also ineffective for large molecules like proteins, peptides, and lipophilic substances. While modifying drug structures to reduce treatment risks shows promise, the current strategies for opening the BBB via this pathway can lead to unintended harm. Future research should focus on better understanding the paracellular mechanism, optimizing drug design, and minimizing side effects to develop safer and more effective treatments for brain disorders.

4.8. Neurotropic Virus-mediated blood-brain barrier delivery

Neurotropic viruses are a special type of viruses which can target neural tissues and infect cells within the nervous system. Recently, researchers have developed several strategies to open the BBB including: employing immune cells as carriers, directly crossing the BBB via transcytosis, utilizing neurotropic viruses to pass through the BBB, or bypassing the BBB by entering the brain via peripheral or sensory nerves [125]. In this case, the idea is to "disable" or to modify the viruses, i.e., to develop neurotropic viruses without pathogenicity, while directing them across the BBB. To date, a few viruses have been designed as vectors for central nervous system transport, such as adeno-associated viruses (AAVs), lentiviruses, and helper-dependent adenoviruses. This delivery strategy may be applied in treating neurodegenerative diseases, brain tumors, and other CNS disorders [126].

Adeno-associated viruses (AAVs) are capable of infecting neurons, astrocytes, oligodendrocytes, endothelial cells, and ventricular plasma cells [127]. They are among the most widely used viral vectors in current research, and are extensively applied in animal models for exogenous gene introduction and expression. Over 100 AAV variants with different functions have been identified. For instance, AAV-

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PHP-B and AAV-PHP. eB have been proved to be able to assist CNS drug delivery and achieve an uniform distribution of drugs in the parenchyma [128]. In the CNS, AAV carriers have the ability to induce long-term expression of exogenous genes, and it has been shown that gene expression induced by AAV vectors can last for more than 6 months in rats, and even more than 6 years in primates and canines [129]. This indicates that AAV, as carriers crossing the BBB, held great potential in treating CNS diseases.

Like most neurons and ECs, lentiviruses, a type of retrovirus, is capable of infecting non-dividing cells. Compared to AAVs, lentiviruses have a larger packaging capacity, thus may carry larger amounts of drugs; lentiviral vectors may also integrate into the host genome, which ensures the long-term stability of gene expression. Lentiviral vectors can be used for direct injection into the CNS or for *in vitro* modification of patient cells, which are then reintroduced into the body for treatment. Currently, lentiviruses have been employed in multiple clinical studies, including a Phase III clinical trial for the treatment of adrenoleukodystrophy (ALD-104; NCT03852498), where autologous CD34+ hematopoietic stem cells (HSCs) from patients were transduced with lentiviral vectors and then reinfused to achieve therapeutic outcomes.

However, despite the many advantages of viral vector drug delivery strategies, challenges still exist for this strategy. For instance, the immunogenicity of viral vectors themselves may trigger immune responses in the host and affect the therapeutic efficiency, and the aggregation in non-target organs may cause peripheral organ infections. In addition, random insertion of viral vectors into the integration site may lead to insertional mutations in host genes, which may trigger the risk of carcinogenesis and lethality. Therefore, there is a pressing need to develop safer alternatives, and researchers are currently developing non-integrating viral vectors through *in vitro* modification and other methods to improve the safety of therapy. With these studies, lentiviral vectors are expected to play an even more important role in the future treatment of brain diseases, offering new possibilities and hope for the treatment of various CNS diseases.

4.9. Other techniques

Besides direct delivery pathways, there is also ongoing research regarding noninvasive methods to increase BBB permeability and monitor BBB integrity. One such approach is transcranial magnetic stimulation (TMS). By stimulating neuronal activity and glutamate release, TMS may increase the permeability of BBB [130]. For example, in a pilot clinical study, TMS improved BBB permeability in 10 out of 15 patients with malignant brain tumors [131]. In another study, *Vazana, U. et al.* have found that when applying low-frequency (1 Hz) repetitive-TMS at a very high amplitude, the BBB permeability transiently increases in the rat cortex [132].

Another non-invasive technique uses near-infrared spectroscopy (NIRS) and functional near-infrared spectroscopy (fNIRS) to monitor real-time BBB integrity. NIRS utilizes the fact that hemoglobin absorbs near-infrared light, where changes in hemoglobin concentration are observed. At the same time, the reflected light attenuation from NIRS can also provide information about regional cerebral oxygen saturation and the balance between oxygen consumption and delivery [133]. Thus, by measuring changes in the absorption of near-infrared light, BBB integrity may be monitored in real-time. Through this technique, researchers may gain implications in optimizing the timing for drug administration.

5. Invasive Delivery Methods

Invasive delivery methods can achieve high concentration of drugs and delivery efficiency. However, several complications take place with the application of invasive methods. Intrathecal delivery, intracerebral injection, and intra-arterial injection are introduced in this section.

5.1. Intrathecal delivery

Intrathecal (IT) delivery is usually used for chronic non-cancerous pain, cancer relevant pain, and spasm therapy. IT injects drugs into the subarachnoid space via lumbar puncture, enabling drugs to diffuse into the CSF.

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The injection devices of IT delivery mainly consist of a pump and catheters. The pump is implanted into the abdominal subcutaneous tissues. Catheters connect to the pump, delivering drugs into intrathecal space. After entering the CSF, drugs diffuse into the pia mater of spinal cord, arachnoid, and the white matter where they either bind with dorsal horn receptors or enter ion channels that engage in injury processing to affect the transmission of pain-sensing signals.

So far, the United States Food and Drug Administration (FDA) has authorized three drugs for IT delivery: morphine, Ziconotide, and baclofen. Morphine and ziconotide are mainly used for neurogenic and nociceptive pain; intrathecal morphine (ITM) is a widely used opioid post-cesarean analgesia [134]. Baclofen is used for muscle cramps caused by multiple sclerosis (MS) and brain paralysis [135].

Advanced IT delivery methods provide solutions for the therapy of PD and neuropsychiatric systemic lupus erythematosus (NPSLE). For example, injecting dopamine into CSF can prevent complications such as peripheral inactivation and stabilize dopamine levels for PD patients [136]. Because of the specificity of NPSLE, IT administration of dexamethasone (DXM) and methotrexate (MTX) has become a useful strategy [137]. Nie et al. found that patients treated with IT recovered better in a short time and have a higher full-recovery frequency than those treated without IT,[137].

In a recent study, IT administration of DXM and MTX is effective for the therapy of granulomatosis with polyangiitis (GPA) involving the fourth ventricle. The effect of the associated injection of glucocorticoids (GCs) and other immunosuppressors is not ideal; however, with the associated injection of GCs and mycophenolate mofetil (MMF), the drug concentration in CNS increases and the brain granuloma gets smaller as well [138].

In contrast to intravenous bolus administration, IT administration has several advantages. First, IT administration can bypass the BBB, allowing macromolecules and antibodies to enter the CNS. Second, direct transport into the canalis spinalis limits the exposure of drugs to the surroundings, stabilizing drugs and leading to lower systemic toxicity. Furthermore, the administration can prolong the drug's half-life since the brain has fewer enzymatic activities. Additionally, the administration is believed to increase the drug concentration around the target via the free molecular exchange mechanism between CSF and brain parenchyma. However, in clinical trials, administrated drug concentrations are affected by numerous aspects such as on multiple aspects such as slow flow of CSF content and increased intracranial pressure (ICP) [139]. Complications caused by IT administration include functional complications (e.g., intellectual and developmental disabilities), physiological complications (e.g., inflection), and other complications (e.g., granuloma) [140].

5.2. Intracerebral injection

Intracerebral injection (ICI) is used for BBB crossing ICI directly delivers drugs to specific encephalic regions via catheters. ICI can be used for both biochemical sampling, passive transport (microdialysis). and positive transport. In microdialysis, perfusate is injected into the probe against the concentration gradient, and micromolecules are collected via a semipermeable membrane for the biochemical sampling [141]. As for positive transport, drugs are injected into specific brain regions via the injection pump. Additionally, the accurate positioning of administration relies on imaging technologies or stereotactic techniques.

Microdialysis is mainly used for the study of metabolic pathways in the brain. The application of microdialysis can monitor the change in the chemical composition of the intracellular fluid in real-time, providing data for drug metabolism and the transmission of neurotransmitters. It can also assess the pharmacokinetics of the brain-administrated drugs [141]. Recently, chimeric antigen receptor (CAR-T) cell therapies have made great progress. For example, Mulazzani et al. found that delivering a small amount of anti-CD 19 CAR-T cell drugs via ICI speeds up and sustains tumor infiltration, successfully removing large tumors in animals treated with the drug [142]. Besides, ICI delivering tigecycline can effectively deal with meningitis caused by colistin-resistant Acinetobacter baumannii, providing lower toxicity and higher selectivity compared with traditional strategies [143].

The latest study has found that the combination of ICI and lipid nanoparticles (LNPs) has great potential for CNS diseases with high biocompatibility. Since the aperture of extracellular space between

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brain cells is around 175-200 nm, it is possible for LNP to cross. Tuma et al. injected LNP mRNA complex into the striatum or hippocampus via ICI, and after analysis of the amount, types, and concentration of the transfected drugs, it was found that a large proportion of the complexes were found in these regions, providing a promising direction in the therapeutic of complicated CNS diseases [144].

ICI can cross the BBB via straightforward injection, ensuring drugs can reach the therapeutic concentration within the target region. Importantly, as for diseases (e.g., drug-resistant bacterial infection, brain metastases) with limited therapies, ICI also offers a new direction. However, ICI has issues with high technical difficulties and high risk such as nerve injury, vascular injury, intracranial hemorrhage, and inflection.

5.3. Intra-arterial Injection

Intra-arterial (IA) can transport a higher concentration of drugs than intravenous delivery to the target areas by direct administration into the carotid or vertebral artery, reducing systemic toxicity [144, 145, 146]. IA has been studied for several decades, and is most commonly used for malignant glioblastomas, [145], retinoblastomas, and primary CNS lymphomas [146].

However, IA delivery does have severe complications. For example, glioblastomas patients exhibit the loss of hearing [147], decrease in eyesight which many related with the change of white-matter [148]. Other complications include numbness, decreased capillary refill, paresthesias, muscle swelling, necrosis, Gangrene, and other adverse symptoms [149].

The promising strategy is to conjugate IA injection with BBB distribution, shuttling large size antibodies such as bevacizumab [150] across the BBB. Osmosis is used for the distribution by hypertonic solutions . These agents result in a reversible distribution of TJ and the shrinkage of the brain endothelial cells by establishing an osmotic gradient [145]. The result shows that the complication such as cognitive impairment is improved via the injection of mannitol [146]. Bradykinin analogs, as another hypertonic solution, increases the permeability of the capillaries of brain tumor cells by stimulating the endogenous vasoactive peptides. Although the experimental results showed that bradykinin analogs can double the uptake of carboplatin IA delivered, this effect only accounts for tumors and the safety of this method remains uncertain [151]. However, the effects of the injection of hypertonic solution is affected by numerous uncertainties such as the injected dose and rate, the individuals' cerebral hemodynamics, and vascular structures[152].

Nanotechnology is used as well. Nanoparticles such as the 20nm trans-activator of transcription-decorated and neutral micelles are capable of bringing drugs across the BBB and target brain tumor cells [153].

Imaging methods are combined to improve the effects of IA injection by monitoring and magnetic targeting. For example, recently, a study of Chengyan Chu et al. injected hyperosmotic mannitol with the guidance of instant magnetic resonance imaging MRI [152](Chu et al., 2021). This method not only renders the users to immediately learn about the distribution territory on the BBB and adjust the injection rate at any time, preventing results of little therapeutic effects or BBB damage occurs [152]. Additionally, MRI is also discovered to increase the absorbance of anti-epidermal growth factor-coated (EGFR) nanoparticles such as dendrimers [154]. Laser interstitial thermal therapy (LITT) and magnetic-resonance-guided focused ultrasound (MRgFUS) can distribute peritumoral BBB [155] [145].

Other BBBO methods for IA delivery include gene therapy and cell therapy. For example, gamma 34.5 and alpha47 genes are injected into carotid artery after the injection of mannitol, and the results show that the mice with cerebral breast cancer injected with the genes have relatively higher survival rate [156].

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Table 4. Comparison of the advantages and limitations of the three discussed invasive methods.

Injection Method	Advantages	Disadvantages	Reference
Intrathecal Injection (IT)	Repeatable drug administration minimally invasive	Spinal cord injury and dorsal root ganglion toxicity The formation of Intrathecal granuloma Epidural or intrathecal bleeding	[135, 140]
Intracerebral Injection (ICI)	Drug-resistant bacteria inflection	Intracranial bleeding High inflection risks	[143]
Intra-arterial Injection (IA)	High drug concentration	Various complications	[145, 146]

6. Conclusions and Future Directions

The paper reviews the existing method for BBB crossing divied into noninvasive methods and invasive methods. For invasive methods, intrathecal delivery, intracerebral injection, and intra-arterial injection are introduced. Invasive methods can transport high concentrations of drugs to target regions while the use of these methods is limited by their in-negligible issues and complications that remain unsolved. On the other hand, noninvasive methods are more commonly used for brain delivery across the BBB. Among these methods, RMT and AMT are the most widely used ones, and Neurotropic Virus-mediated blood-brain barrier delivery is considered to be a promising delivery method. Although the study toward noninvasive methods is relatively thorough, further exploration to improve the selectivity, drug loading capacity, stability and other properties of these methods is needed.

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