Consensus Review on Strategies to Improve Delivery Across the BBB Including Focused Ultrasound

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Key points:

- The BBB is a major obstacle in CNS chemotherapy delivery, which has been mitigated by utilizing natural transport mechanisms or disrupting the BBB.
- New technologies have emerged as methods to temporarily disrupt the BBB and potentially provide access for non-invasive biopsies and CNS delivery of chemotherapy.

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Abstract

Drug delivery to the central nervous system (CNS) has been a major challenge for CNS tumors due to the impermeability of the blood brain barrier (BBB). There have been a multitude of techniques aimed at overcoming the BBB obstacle aimed at utilizing natural transport mechanisms or bypassing the BBB which we review here. Another approach that has generated recent interest in the recently published literature is to use new technologies (Laser Interstitial Thermal Therapy, LITT; or Low Intensity Focused Ultrasound, LIFU) to temporarily increase BBB permeability. This review overviews the advantages, disadvantages, and major advances of each method. LIFU has been a major area of research to allow for chemotherapeutics to cross the BBB which has a particular emphasis in this review. While most of the advances remain in animal studies, there are an increasing number of translational clinical trials which will have results in the next few years.

Keywords : Low intensity focused ultrasound, blood-brain barrier, neuro-oncology, drug delivery, MRI-guided focused ultrasound

Introduction

Despite decades of advances in understanding the genetics and biology of primary malignant brain tumors, there has been little success in improving the patient survival via conventional routes of therapeutic delivery (e.g., oral, intravenous formulations).[1] One of the key challenges in the delivery of therapeutics to the central nervous system (CNS) is the presence of the blood-brain-barrier (BBB) and blood-cerebrospinal fluid-barrier.[1, 2] It has been estimated that these barriers prevent more than 98% of potential neurotherapeutics from reaching the CNS, and consequently much effort has been devoted to finding ways to either change the physical/chemical properties of drugs to make them more brain penetrant, directly deliver therapeutics into the brain or make the BBB more permeable to existing therapeutics.[1-5] This review will focus on some of the more recent methods to open the BBB to systemically delivered therapeutics or bypass it for direct delivery of therapeutics to brain tissue (**Figure 1**) and the current state of the research in each modality (**Table 1**).

Blood-Brain-Barrier

Anatomically the BBB consists of cerebral endothelial cells, pericytes, astrocytes, and basement membrane.[6] These cells are non-fenestrated and linked by tight junctions to highly regulate molecular transport. The tight junctions have been a primary hurdle for disruption of the BBB.[7] An intact BBB limits entry of 98% of small molecule drugs and often near 100% of large molecule drugs.[1, 2] Trauma, inflammation, and tumors can disrupt the integrity of the BBB; therefore, in patients with cancer, it is referred to as the blood-brain tumor barrier (BBTB) which is a more heterogenous category.[4] The tenets of these naturally occurring BBB breakdown have been exploited to treat central nervous system (CNS) cancer. Gliomas, however, have a varying degree of BBB breakdown with many having anatomically intact (non-contrast enhancing) regions that do not allow CNS penetration of chemotherapeutics.[8] Even if a drug is able to permeate contrast enhancing tumor tissue, it is unlikely to reach the diffuse tumor cells that rest behind the intact BBB.

There are three major mechanisms that allow for drug molecules to cross the BBB: (1) increasing BBB permeability, (2) bypassing the BBB via direct brain delivery and (3) utilizing natural transport mechanisms (**Figure 1**). Many have postulated the ideal properties for a drug to cross the BBB including small molecular weight <500 g/mol, lipid soluble, electrically neutral, and weak bases.[3] Altering chemotherapeutic agents or packaging them into BBB-permeable means has been one approach to overcoming the BBB problem, often with nanoparticles used in conjunction with another means of BBB disruption.[9, 10] Other approaches have involved the use of targeted pharmacological agents or high-osmolarity infusions to transiently open the BBB,[11] but more recently there has been an emphasis on increasing BBB permeability via the application of other technologies used primarily to create lesions in the brain. Specifically, these technologies are low intensity focused ultrasound (LIFU) and laser interstitial thermal therapy (LITT). These two procedures are unique in their use of MR thermometry to protect eloquent areas of the brain while creating BBB breakdown in a targeted manner **(Figure 2)**. This review summarizes what is known about the use of these new modalities to overcome the BBB in treating CNS tumors.

TRANSPORT-MEDIATED SYSTEMS TO ENABLE BBB PENETRATION

Utilization of endogenous transport mediated systems has emerged as an active area of pre-clinical research.[12] These efforts exploit native cellular biology using novel techniques such as genetically modified ligands on the surface of drug-loaded nanoparticles or antibodies with specific receptors expressed on brain endothelial cells.[13] Each of the three transcytosis methods discussed here are provided in brief as they are currently largely limited to animal models and have a very broad field of research that is too extensive to be included in this overview.

Receptor-mediated transcytosis (RMT)

Amongst transport-mediated systems, receptor-mediated transcytosis (RMT) is currently the most utilized mechanism in research for CNS penetration. RMT is a naturally occurring process where macromolecules bind to receptors that initiates uptake across the BBB.[14] There are a multitude of research paradigms in this field; for example, one approach uses murine 83-14 monoclonal antibodies to human insulin receptors. [15, 16] In a primate model, humanized insulin receptor monoclonal antibodies were rapidly taken up in the Rhesus monkey brain via a receptor-mediated transcytosis pathway and proposed for human trials.[17] Similar efforts are ongoing with many animal model publications utilizing a variety of receptor targets often including transferrin and low-density lipoprotein receptor-related protein in addition to insulin. [18, 19] Angiopep2 is a specific oligopeptide that utilizes a low-density lipoprotein receptor-related protein 1 and the RMT process that has demonstrated advancements in CNS drug delivery across many applications including stroke, epilepsy, tumor, brain injury, and neurodegenerative diseases.[20, 21] While receptor-mediated transcytosis deserves a larger and more extensive review, it's applicability has just recently reached clinical trials which are hopeful to show results in the next decade. The historical use of RMT for non-neurological indications has allowed for a fast growth of RMT research in CNS tumor therapy through BBB penetration as well as in non-tumor applications of BBB disruption.[14, 22, 23] The use of target transport vehicles such as transferrin receptors have been a promising focus with multiple clinical trials that have been approved by the United States Food and Drug Administration (FDA) for other indications beyond BBB penetration.[22-26]

Adsorptive-mediated transcytosis (AMT)

Adsorptive-mediated transcytosis (AMT) acts to circumvent the BBB by utilizing the electrostatic interactions to facilitate transcytosis between the naturally negatively charged luminal and abluminal membrane surfaces of the BBB and positively charged proteins.[27] The idea emerged from studying polycationic proteins such as protamine which can bind to endothelial cells and subsequently penetrate the BBB. This was then explored with many other substrates including histone, avidin, several agglutinins, monoclonal antibodies, and many basic peptides.[28] This mode of transport across the BBB is generally considered unidirectional, and is mediated by clathrin-dependent endocytosis.[29]

Most investigations into AMT have also been preclinical, using either animal models or *in vitro* studies of live human cells. One mode of drug delivery using AMT involves conjugating a target drug to a cationized molecule. Popular options include the polysaccharide chitosan and albumin.[30] Albumin is particularly promising because albumin-binding proteins and potential transporters have shown increased expression in glioblastoma.[31] Specifically, in an *in vitro* study of animal brain endothelial cells, doxorubicin conjugated to a carrier and cationized albumin had a 10 times higher permeation than plain doxorubicin, and a 1.5 times higher penetration when compared with the same formulation without the cationized albumin.[32]

In addition to conjugation with a cationized ligand, a cationic polymeric core may be fashioned to act as a reported "Trojan horse" to deliver therapeutic agents. This mechanism may condense and protect nucleic acid from endosomes by taking advantage of their proton buffering capacity.[33] In an *in vitro* and *in vivo* animal study, Park et al. showed successful and enhanced delivery of a rabies virus glycoprotein across the BBB in an evaluation of RNA therapeutics for Alzheimer's disease.[34]

AMT does have certain limitations. Low lipid solubility of the entire compound will hinder permeation across the BBB.[35] There are also mixed concerns regarding cytotoxicity associated with cationic surfaces compared with neutral surfaces.[36] However, at least one study found no difference in rate of cell membrane damage between normal and cationized albumin.[37] The possible risk for cytotoxicity warrants further investigation. Meanwhile, the mechanism of electrostatic interaction that AMT relies on is nonspecific and may be susceptible to other reticuloendothelial systems such as those found in the liver or lungs.[36]

Overall, there is momentum in AMT research as a means for drug delivery across the BBB. While clinical trials have not yet produced extensive results, some studies have utilized a combination of therapies including AMT and nanoparticles, along with ultrasound mediated blood brain barrier disruption (see below), to increase drug penetrance in the CNS (e.g. NCT04528680).[38, 39] Clear demonstrations of clinical efficacy are currently lacking and the next decade will determine whether this technique will translate to FDA-approved therapeutics.

Solute carrier-mediated transcytosis

Solute carrier-mediated transcytosis (SCMT) utilizes native nutrient transporters much like RMT. However, SCMT generally uses larger transmembrane carrier proteins and nutrient channels specific to a certain solute, allowing for active transport of the solute across the membrane using ATP hydrolysis. Examples of solute transporters and their respective solutes include glucose transporter type 1 (GLUT1) and glucose, monocarboxylate transporter 1 (MCT1) and monocarboxylic acid (eg. lactic acid) and large amino acid transporter 1 (LAT1) and large amino acids such as phenylalanine and its derivatives. This natural process can be used to facilitate drug delivery across the BBB by combining drugs and solutes or other natural analogs that mimic the solutes for which the transport channels have high affinity.

Many SCMT conduit transporters have been extensively studied in preclinical and animal models.[36] In particular, GLUT1 and LAT1 possess high enough transport capacity to be potential modes of clinically meaningful drug delivery across the BBB.[40, 41] LAT1 is abundantly expressed in both the abluminal and luminal surfaces of the BBB and has a 100-fold higher expression level in capillary endothelial cells in the BBB than in peripheral tissues such as the retina, intestines, and placenta.[41] LAT1

is also preferentially expressed in high grade glial tissue. Kobayashi et al. detected expression of LAT1 at high, moderate, or low levels in 75% of glioblastoma (grade IV astrocytoma) and anaplastic astrocytoma (grade III) but only at low levels in only one of three diffuse astrocytoma (grade II) and was also undetectable in three samples of nonneoplastic tissue.[42]

Furthermore, SCMT is also invoked in the mechanism of action used by some common drugs, including L-DOPA, gabapentin, baclofen, and melphalan – all of which mimic the natural substrates of LAT1.[43-46] However, despite the potential of SCMT for enhancing drug delivery across the BBB, clinical trials utilizing SCMT for chemotherapeutic drug delivery have yet to produce any meaningful results.[36] For all type of transcytosis, it is difficult to provide an exhaustive review here as many of the studies now involve multimodal treatment therapies and are often not clear in the type of transcytosis being utilized. While we have provided several representative cases, individual reviews are needed for each specific category.

Nanoparticles

The BBB represents only part of the issue of CNS drug delivery as the drug can be limited to the extracellular space in a hydrophobic and electrostatically charged medium.[47] Brain-penetrating nanoparticles (NPs) are coated in polyethylene-co-glycol (PEG) which has the advantage of superior stability in the bloodstream but has reduced exchange through the BBB, which makes it a great option in combination with low intensity focused ultrasound (LIFU) or convection enhanced delivery (CED), a form of direct brain delivery.[47-49] Organic NP have been also explored from lipids which have higher biocompatibility and natural degradation processes.[43]

Polymetric NPs have been a rising field of BBB access in which a therapeutic agent is encased in or bound to an NP for CNS penetration via adsorptive-mediated, receptor-mediated, or carrier-mediated pathways.[6] These NPs have a wide array of compositions. Poly(alkyl cyanoacrylates) (PACA) NPs were some of the earliest, first developed in 1972, now with a known low toxicity and a good comprehension of its degradation by esterase, a process whose duration can be controlled by modifying the alkyl side chain length.[6] Other copolymers are Poly(lactic-co-glycolic acid) (PLGA) and Poly-ϵ-caprolactone (PCL). PLGAs, PACAs and PCLs have been approved clinically in several different drug delivery systems and tested in animal models for CNS delivery but not yet completed phase III clinical trials for CNS delivery.[6] Polyamidoamine dendrimers (PANAM)s are layered structures that can entrap drugs via hydrophobic cavities, usually smaller than 15 nm. Synthetic polymers have advantageous shape and size but can be restricting due to their cost and toxicity profiles. Thus, natural NPs have been explored including chitosan, a biodegradable cationic linear polysaccharide, and alginate, an anionic linear unbranched polysaccharide extracted from brown seaweed.[6]

NP strategies for BBB-crossing have targeted specific size, shape, ligand-density, surface chemistry, and lipophilicity.[10] For nanoparticles, the smaller sizes have been associated with higher BBB penetration but also with faster renal clearance for those smaller than 5 nm.[50] Most nanoparticles range between 30-100 nm.[10] Morphology varies significantly with some studies suggesting higher BBB penetration with rod shapes vs. sphere shape, but many studies hypothesizing higher penetration capacity with more complex shapes such as wires, wreaths, rings or plates.[9] Surface alterations such as increased ligand density, particularly studied APO-E protein and coated particles, such as PEG and poly(lactic acid) coating have been shown to increase BBB penetration.[10]

NP advances have been diverse and extensive in the past decade. Much of the recent literature on crossing the BBB with NPs has been in conjunction with interventions to increase BBB permeability. Michael et al. and Kievit et al. both demonstrated multisystem interventions to improve drug delivery with CED, tumor targeting ligands, radiotherapy and immunotherapy.[51, 52] There are many reviews on NPs alone. While we include mention of NPs here, an exhaustive review of NP role in achieving BBB penetration is beyond the scope of this review.

Similar to NPs, blood exosomes, naturally created vesicles from cells, have been utilized as a tool for delivering drugs. Zhan et al.[53] demonstrated the delivery across the BBB of siRNA and metformin within exosomes preferentially accumulating in human derived GBM cells that expressed polymerase 1 and transcript release factor. Exosome delivery is immunologically attractive over viral vectors as it has a low likelihood of triggering an immune response and do not cause any toxicities that can be seen with nanoparticles.[2] This is a relatively new area for *in vitro* research that will likely be explored more extensively in the next couple years.

INCREASING BBB PERMEABILITY

Low Intensity Focused Ultrasound (LIFU)

Magnetic resonance imaging guided focused ultrasound (MRgFUS) uses ultrasonications and MR thermography monitoring to cause tissue disruption through microimplosions, resulting in cavitations that disrupt tight junctions on the endothelial surface.[54, 55] This mechanism of action has been known for decades and utilized in the treatment of tumors across the human body as well as a more recent application utilizing a high intensity form of ultrasound to create permanent intracranial lesions.[55, 56] High-intensity focused ultrasound (HIFU) is FDA-approved for thalamotomy for essential tremor utilizing 650-kHz that produces temperatures of 56°C that causes coagulative necrosis.[57] While BBB breakdown has been demonstrated in HIFU since 1990, further refinements have discovered BBB breakdown without damage to adjacent neural tissue with lower frequencies.[58] From these discoveries, further research in low intensity focused ultrasound (LIFU) led to a recognition that lower intensities are not ablative to neurological tissue but still are useful for creating BBB disruption.[59] For some technologies that perform LIFU, the intensities are too low to produce cavitation-induced microbubbles; instead, the microbubbles must be provided, typically via IV injection. Other technologies do not require the use of infused microbubbles (see below). In either case, temporary BBB breakdown occurs by intravascular microbubble cavitation-induced tight junction disruption and the consequent paracellular transport that permits passage of molecules for ~ 4-8 hours.[58, 60] However, the BBB mechanism of disruption is dependent on the intensity of the sonication and not yet fully understood.[61]

Both LIFU and HIFU work by applying a silicone barrier to the head, sealing cooled gasless water in the transducer cavity; the head is often shaved to prevent thermal damage and hair interference. Sonications have predominantly been applied with passive cavitation detectors and MR thermometry to monitor response and prevent injury to adjacent structures, however several newer ultrasound delivery devices are utilizing neuronavigation without MR thermometry given the lower risk of neural injury with low intensity ultrasound.[62-64] While HIFU has been applied in many human clinical trials predominantly for movement disorders, intracranial application of LIFU is just now being utilized in clinical trials.[65, 66]

The clinical significance of LIFU for BBB disruption has been demonstrated in numerous animal models showing enhanced delivery of systemically administered agents to intracranial tumors including temozolomide, doxorubicin, paclitaxel, methotrexate, cisplatin, bevacizumab and carmustine for a range of tumors.[34, 62, 67-75] In addition to chemotherapy agents, other agents including targeted therapies, immunotherapies and large molecules have been shown to have higher CNS concentrations after LIFU BBB.[47, 76, 77] In these animal models, the BBB disruption occurs immediately and resolved within eight hours, while clinical studies suggest this BBB repair may occur within the first hour.[62, 78] The true range of the BBB disruption may be patient- or pathology- specific and further studies to define the characteristics are still to be determined.

While recognition of the effect of ultrasound on tissue disruption has been present for decades, the application of LIFU to produce BBB breakdown was first introduced by McDannold et. al in rabbits in 2006.[79, 80] After further investigation of this approach in numerous small rodent models,[81-86] Rezai et al. demonstrated BBB disruption in six patients with Alzheimer's disease (AD) reporting the safety, feasibility, and reversibility of *in vivo* human use of LIFU for BBB disruption.[87] BBB disruption was utilized *in vivo* for drug delivery in AD and Parkinson's disease (PD) prior to its application for tumor drug delivery.[88, 89] This has been an active area of research with recent publication showing reduction in amyloid and progression of disease in patients with dementia.[90, 91] LIFU use in tumor drug delivery has been applied in a multifactorial manner with nanoparticles to enhance drug delivery in a number of models, with one of the earliest by Wang et al. demonstrating enhanced drug deliver to tumor cells across the BBB.[92] While there are no large scale published human *in vivo* studies on BBB disruption for tumor drug delivery, there are a number of clinical trials for this topic as well as BBB disruption for liquid biopsies of central nervous system (CNS) tumors.[93]

Ultrasound has been a rich area of exploration beyond only BBB breakdown with liquid biopsy and sonodynamic therapy. Liquid biopsy enhancement with LIFU has been demonstrated in animal models in which LIFU opens the BBB allowing for tumor makers to be identified from the venous system.[62, 77, 94] Sonodynamic therapy, which couples LIFU with 5-aminolevulinic acid (5-ALA) triggers production of high volume of reactive oxygen species that act to kill tumor cells.[95] This approach has already been implemented for high grade glioma animal models with promising overall survival outcomes.[96, 97] Sonodynamic therapy has also demonstrated some BBB breakdown effects similar to LIFU that may be a popular area of exploration in the upcoming research.[98]

Utilizing a similar mechanism of action of producing cavitations via ultrasound, an implantable ultrasound device that can be activated on-demand has been developed with the goal of bypassing technical challenges that arise with noninvasive ultrasound delivery to deliver ultrasound without needing to transverse higher density areas of tissue not within the target.[63, 64] While this method is more invasive, requiring a craniotomy to create a window through the skull to deliver ultrasound, this device remains in an investigational phase at this time.[63] This implanted device uses a similar process to LIFU called low intensity pulsed ultrasound (LIPU), which provides an intermittent version of ultrasound that provides a more diffuse opening of the BBB that does not require MR guidance.[99, 100] LIPU is advantageous in not requiring an MRI, and trials have shown significant CNS drug uptake and speedy BBB recovery. However, the effects of diffuse LIPU to the brain are not fully understood.[39, 101-103] While MR thermometry is an important tool for safe treatment in HIFU, other methods of neuronavigation have been utilized to allow for accurate BBB disruption without the need for MR during ultrasound delivery (e.g., NaviFUS).[104]

Laser Interstitial Thermal Therapy (LITT)

Laser interstitial thermal therapy (LITT) has existed for decades but its use intracranially for tumor treatment is relatively new. LITT has been used in a tissue ablation manner since 1966, but was not approved for neurosurgical ablation until 2007.[105] LITT utilizes optical radiation from a laser probe to heat surrounding structures. The probe is placed stereotactically with submillimeter accuracy, and MR-thermography is used to monitor temperature elevation in nearby non-target tissue, allowing for safe and accurate target ablation.[105, 106] LITT has been utilized for various pathologies including epilepsy, cancers, radiation necrosis, cavernous malformation, and other benign intracranial lesions.[106] The role of LITT in exclusively disrupting BBB has not been extensively explored, but several studies have demonstrated its potential role in applied chemotherapeutic agents after utilizing LITT for tumor ablation.[105, 107] Specifically, there are several studies showing improved glioma outcomes in patients who underwent LITT, in which the authors speculate the BBB breakdown may be a contributing factor.[108, 109]

Other studies have demonstrated radiographic and laboratory evidence of BBB disruption. Leuthardt et al. demonstrated BBB disruption in fourteen patients with high grade gliomas by for up to four weeks post-LITT by measuring peripheral blood CNS biomarkers and radiographic evaluation of BBB disruption.[110] Similar mouse models have demonstrated improved survival with doxorubicin in combination with LITT is now being explored in two clinical trials.[111-114] Further clinical benefits of LITT have been postulated as eliciting an immune response to augment immunotherapies.[115]

Superselective intraarterial cerebral infusion (SIACI)

While there are a number of external devices aimed at increasing BBB permeability, there are also pharmacologic means such as superselective intraarterial cerebral infusion (SIACI). Intraarterial (IA) delivery of medications for CNS tumors was initially utilized to reduce systemic side effects, but over time IA hyperosmolar therapies demonstrated a reversible disruption of BBB tight junctions.[116] A number of chemotherapeutic agents including bevacizumab, temozolomide and cetuximab have been utilized in conjunction with mannitol leading to higher drug concentration in the tumor.[117] Hyperosmotic mannitol has been utilized to create an osmotic BBB disruption via transient shrinking of the endothelial cells and opening of the tight junctions.[7, 118] This has even been further refined with endovascular local delivery of IA medication via distal arterial branches to the tumor. [119] Mannitol has been predominantly explored as the drug delivered to incite BBB breakdown with currently dozens of clinical trials exploring its applications.[116, 119]

DIRECT DELIVERY

Multiple approaches have been taken to directly administer therapeutic agents to the CNS: 1) intrathecal (into the cerebrospinal fluid, CSF), 2) intracavitary (via passive release), 3) injection (stereotactic or hand injection via a surgical cavity), and 4) convection enhanced delivery (transcranial over hours to days). While intrathecal delivery has been shown to be clinically effective for cancer cells that spread to the CSF,[120] it does not produce pharmacologically meaningful distribution into the brain parenchyma and the intrathecal and intercellular spaces function as distinct compartments with poor exchange kinetics.[121, 122] The intracavitary approach has been successful as demonstrated by FDA-

approval of carmustine wafers in the mid-1990's.[123] However, the clinical benefit was small and these studies were done prior to the advent of an oral chemotherapy (temozolomide), which showed greater efficacy for treating GBM.[124-126] The kinetics of intracavitary delivery are challenging in that they rely on the passive mechanism of diffusion and the exponential decay in concentration as a function of distance. With this delivery mechanism, the concentration of agent at the surface of the cavity is limited by toxicity and there is an exponential decay in the concentration which can lead to subtherapeutic concentrations within millimeters of the cavity wall. Currently, the intracavitary approach provides a short duration of treatment as the delivery substrate becomes depleted of drug within weeks.

Intraoperative Delivery

Intraoperative drug delivery has been explored as a means of bypassing the BBB and largely as an adjuvant to surgical resections. One of the most well-known was carmustine wafers which in 2 prospective randomized studies showed a small survival benefit but which was associated with high complications rates.[125, 127, 128] These results led to FDA approval of this new therapy; yet, the relatively small benefit was in the pre-temozolomide era and the combination of radiotherapy and temozolomide supplanted the use of carmustine wafers. Nevertheless, they remain an important proof of principle of the potential benefit associated with loco-regional therapeutic delivery. Although carmustine wafers may be the most well-known study, many other intraoperative medications have reached clinical trials. Vocimagene amiretrorepvec with flucytosine reached phase 2 trials for post-surgical resection cavity injection, but did not show an overall survival benefit in high grade gliomas.[129] Other studies have focused on injecting therapeutics directly into the tumor after a biopsy without any surgical debulking.[130] Specifically, the use of recombinant viruses and combination therapies have shown potential in high grade glioma survival rates.[130, 131] The use of oncolytic virus-based cancer immunotherapy has been extensively studied and are now being applied to intraoperative delivery and in conjunction with other immune system inhibitors.[132]

Convection Enhanced Delivery

Convection Enhanced Delivery (CED) is a technique of delivering drugs by bypassing the BBB directly to the CNS via a catheter.[133, 134] Differing from intrathecal delivery, these catheters are directly implanted into parenchyma. This method was pioneered at the National Institute of Health by Edward Oldfield's group in the early 1990s.[133] CED specifically uses bulk flow via a pressure gradient rather than diffusion, which allows for drug delivery independent of molecular weight or diffusivity.[134, 135] The interstitial pathways allow for convection transport independent of molecule size, but the pial surfaces can act as barriers limiting flow.[133] However, CED has its own limitations including reflux along the implanted catheter affecting intended dose or flowing around catheter, white matter edema, air bubbles, challenges in ratio of infusion volume and flow rate. Since 1997 there have been over 20 completed trials involving CED for gliomas.[133, 134] The PRECISE trial is the only phase III trial to have been completed and it compared CED of IL13-PE38QQR exotoxin to use of carmustine-impregnated wafers for patients with recurrent GBM undergoing an intended complete resection. PRECISE did not demonstrate any overall survival benefit but did show a PFS favoring CED by >5 months.[136] Beyond interleukin receptor

targeting agents, topotecan, paclitaxel, carboplatin, and nonpathologic recombinant poliovirus have been trialed with mixed results.[133, 137] While the current use of CED involves external catheters to mechanical pumps, future directions may involve a semi-permanent implantable, refillable system.[134]

There are several significant challenges that have been recognized to limit the success of this delivery method. While initial clinical trials focused on the delivery of existing or novel therapeutic agents, it was assumed that any existing catheter that is used in neurosurgical procedures would be sufficient to deliver into tumor or tumor infiltrated brain. Also, there was no direct method to visual actual delivery into brain tissue – indirect imaging of the effects of delivery, via MRI, have intrinsic limitations. Unfortunately, it was not until multiple trials failed to show clinical impact that the use of co-infused imaging tracers made it obvious that the so-called "off the shelf" catheters have not produced reliable delivery. Subsequent development of catheters with tip designs that were optimized for delivery into brain tissue led to small studies that showed successful and predictable drug delivery to targeted areas in the brain.[134, 135, 138]

CED has been utilized to test the efficacy of many known immunotherapies and gene therapies. While many of the studies are still in phase 1 trials, there are several promising publications after in human use via CED.[138] OS2966 recently published a promising phase 1 utilizing humanized and de-immunized monoclonal antibodies targeting CD29/B1 integrin via CED. MDNA55, an immunotherapy aimed at interleukin 4 receptor, has demonstrated acceptable safety profile and tumor control that is now being utilized in phase 3 trials.[139] Additionally, CED is being utilized as adjuvant therapy by implanting into resection cavities in a similar paradigm studied with intraoperative injections but with a more prolonged therapeutic delivery such as cintredekin besudotox, which showed promising results in phase 1.[140]

While the currently used, CED-optimized devices have been shown to provide more reliable delivery, they all have the limitation of being implanted only temporarily (hours to days prior to removal). For infiltrative gliomas, however, where there is interest in a wider spectrum of therapeutics, it is unclear whether a single treatment session via CED will be adequate to produce a durable clinical response. In contrast, there are currently multiple studies involving viral vectors engineered to provide gene therapy for neurodegenerative diseases.[141-143] In that setting, virally-mediated gene delivery is performed to a small (~1 cc) target, which can be completely covered during a single surgical episode; repeat therapeutic administration may not be required in that setting.

A fully implanted CED system, which could be intermittently accessed over a prolonged period of time via a skull-implanted external port, was used in a UK-based clinical trial in patients with recurrent glioblastoma. While this small, single armed trial showed that the approach was safe, and the study reported encouraging clinical outcomes, there was no imaging of delivery performed (via a tracer) to indicate the extent of distribution of the administered agents.[141, 144-147] Currently, the device that was used for that study is not commercially available for more widespread use.

Intranasal drug delivery

Intranasal drug delivery for CNS penetration was first published in the late 1980s with a proposed mechanism of BBB bypass through the olfactory and trigeminal nerves.[43, 148, 149] Intranasal route to the CNS is advantageous in that the nasal cavity rapidly absorbs drugs, does not require sterile delivery, painless and convenient.[43, 149] However, the studies on intranasal CNS drug delivery have not yet shown reliable reproducibility or not reached phase 3 trials.[43, 150] Yet, there are recent studies complimenting intranasal drug delivery with chemotherapies in nanocarriers to enhance these methods.[151-153]

Conclusions

The blood brain barrier is fortress preventing drug delivery to CNS tumors. Over the past thirty years, the means of bypassing or weakening the BBB have expanded significantly. While intranasal delivery and nanoparticle carriers continue to show improvements, more recently LITT and LIFU have made fast strides towards clinical trials. The various transcytosis methods are drastically growing from research in other non-CNS fields and area advantageous in that no invasive procedure is required but the time to clinical application may not be in the near future. While SIACI, CED, and LITT are all minimally invasive procedures they still have associated surgical risks. With many different therapies providing marginal results, researchers are now commonly combining these various methods to increase the efficacy which will be an important topic to follow in the future. While there are a wide array of topics being studied for CNS drug delivery penetration, this summary aims to provide a concise overview.

FIGURES

Figure 1: Overview of mechanisms to overcome BBB for treating CNS tumors

Figure 2: Procedures utilizing MR thermometry to create BBB breakdown

Table 1: Techniques and Technologies for Enabling Treatment Delivery for CNS Tumors across the BBB

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Table 1: Techniques and Technologies for Enabling Treatment Delivery for CNS Tumors across the BBB

Technique	Technology	Types of Agents Delivered	Current Status	Publications*	Representative Clinical Trials**
Transcytosis	Receptor-Mediated	Biologics	Primarily Animal Models	21	NCT03053089 NCT03071341
	Adsorptive-Mediated	Biologics	Primarily Animal Models	38	NCT04528680 NCT00313599
	Solute Carrier-Mediated	Drugs, Biologics	Primarily Animal Models	No representative published human trials found	None actively recruiting
Blood-Brain-Barrier Disruption	LIFU	Drugs, Biologics	Investigational	57, 66, 73, 74, 87	NCT05733312 NCT05317858 NCT05630209 NCT05615623 NCT05293197 NCT04021420
	LITT	Drugs, Biologics	Investigational	107, 110, 113, 114	NCT03277638 NCT04181684 NCT04699773
	SIACI	Drugs, Biologics +/- mannitol	Investigational	117, 119	NCT05773326 NCT05271240 NCT02861898
Direct Brain Delivery	Carmustine Wafers	Carmustine	FDA approved	123	NCT04222062 NCT05083754
	Direct Injection	Drugs, Biologics	Investigational	129-131	NCT00479765
	CED	Drugs, Biologics	Investigational	136, 138-140	NCT03500991 NCT03638167 NCT04185038

**This list is meant to be a representative sample of the manuscripts published but is not extensive given there are many instances in which multiple modalities are utilized and in several instances the type of transcytosis is not clearly defined.*

***Some categories have >10 ongoing clinical trials and therefore the most relevant and active NCT's were included as a representative sample of ongoing research.*

Abbreviations LIFU: Low-Intensity Focused Ultrasound, LITT: Laser Interstitial Thermal Therapy, SIACI: Superselective IntraArterial Cerebral Infusion, CED: Convection Enhanced Delivery, FDA: Food & Drug Administration, NCT: National Clinical Trial

Figure 1

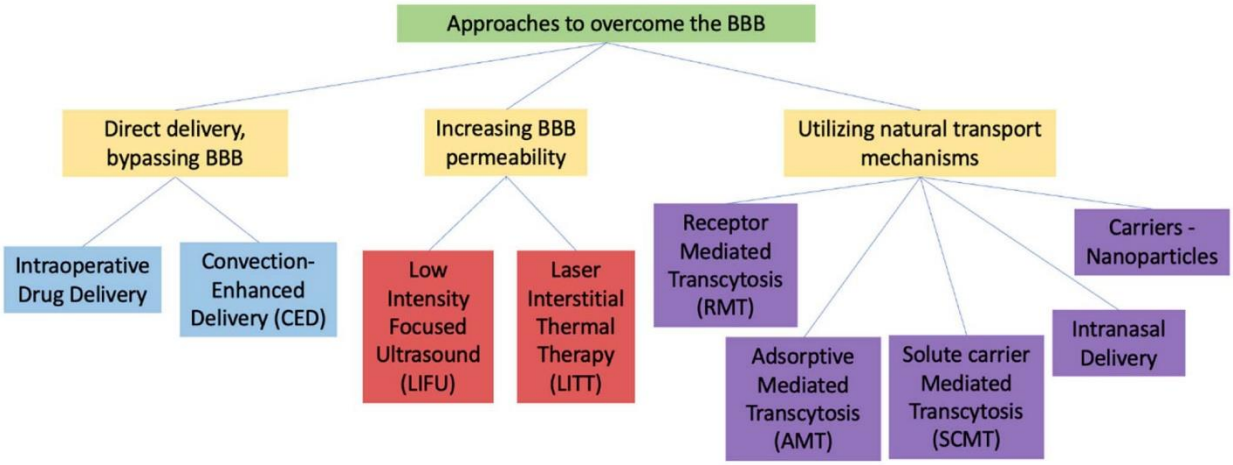

Figure 2