ACS Chemical Neuroscience

Review

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Noninvasive and Targeted Drug Delivery to the Brain Using Focused Ultrasound

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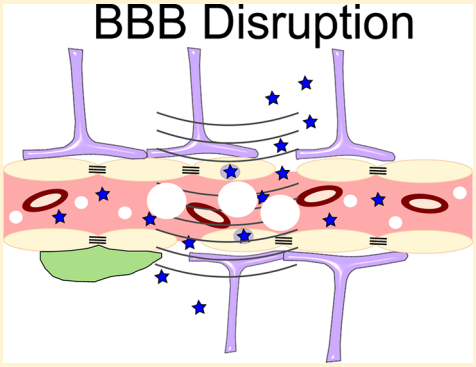
ABSTRACT: Brain diseases are notoriously difficult to treat due to the presence of the blood-brain barrier (BBB). Here, we review the development of focused ultrasound (FUS) as a noninvasive method for BBB disruption, aiding in drug delivery to the brain. FUS can be applied through the skull to a targeted region in the brain. When combined with microbubbles, FUS causes localized and reversible disruption of the BBB. The cellular mechanisms of BBB disruption are presented. Several therapeutic agents have been delivered to the brain resulting in significant improvements in pathology in models of glioblastoma and Alzheimer's disease. The requirements for clinical translation of FUS will be discussed.



KEYWORDS: *Focused ultrasound, blood-brain barrier, drug delivery*

Brain diseases, including psychiatric disorders, neurodegenerative diseases, and cancer, are among the most prevalent diseases worldwide. It has been estimated that 35% of all disease burden is attributable to brain disorders.[1] Despite advancing research, which better understands the etiology and underlying mechanisms of disease, most of these diseases do not have effective treatments and essentially no cures exist.

Designing therapeutic agents for the brain is very challenging. The brain is a well-protected organ, completely encased by the skull, making surgical access difficult and direct application of drugs impractical. However, perhaps the most limiting factor to successful treatment of brain disease is the blood-brain barrier (BBB) which prevents access of ~98% of current pharmaceutical agents to the brain when delivered intravenously.[2]

THE BLOOD-BRAIN BARRIER (BBB)

The BBB is a specialized structure between the cerebral capillaries and the brain parenchyma that is relatively impermeable except for a selection of very small (<400 Da), lipophilic compounds.[3] The BBB is different from the barriers between the peripheral vasculature and other organs in the body due mainly to the presence of tight junctions between adjacent endothelial cells.[3] Cell adhesion molecules, most notably claudins and occludins, connect the endothelial cells together to create the tight junctions. The intracellular domains of the proteins are anchored to the cytoskeleton and the extracellular domains form homodimers with proteins on adjacent endothelial cells. These independent tight junctional proteins work in concert to make the endothelial cellular layer impermeable to fluid thereby limiting paracellular transport mechanisms.[3] In addition, there are a decreased number of transport vesicles in endothelial cells of the BBB, thereby limiting transcellular transport. The endothelial cells are further supported by a basal lamina and a complex cellular system of astrocytes, pericytes, microglia and neurons which function together as the BBB[4] (Figure 1).

The intact BBB is imperative for maintaining the delicate environment required for proper function of the neuronal circuitry. It regulates ion concentrations within narrow ranges and prevents the access of neurotoxins, immune cells. and

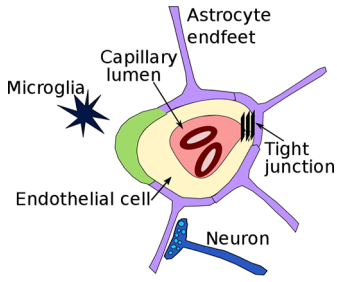


Figure 1. Components of the blood-brain barrier (BBB). The BBB exists between the cerebral vasculature and the brain parenchyma. Transmembrane proteins connect adjacent endothelial cells to each other, creating tight junctions and making the endothelial cell layer impermeable to water. The endothelial cells are supported by a layer of basal lamina, pericytes, and astrocytes. The astrocyte endfeet provide a direct link between the cerebral capillaries and the neurons.[5]

Received: October 29, 2012
Accepted: January 21, 2013
Published: January 21, 2013

Method	Advantages	Limitations
Novel drug design	Able to cross BBB Non-invasive: drugs can be delivered orally or i.v.	Expensive to develop Inefficient drug delivery May have adverse systemic effects
Drug modification (carrier molecule)	Able to cross the BBB Non-invasive: drugs can be delivered orally or i.v.	Expensive to develop Inefficient drug delivery May have adverse systemic effects
Intranasal delivery	Non-invasive: drugs are delivered through the nasal passages	Inefficient drug delivery Non-targeted: may affect other brain regions
Surgical intervention	Targeted to affected brain regions Efficient drug delivery	Invasive
Chemical disruption of BBB (mannitol)	Transient – increased permeability lasts ~30 min	Non-targeted: entire BBB is affected Invasive – requires intra-carotid injection
Focused Ultrasound	Non-invasive: bubbles given i.v. Targeted to a specific brain region, Transient: BBB closed within 6 hrs Efficient drug delivery	Requires clinical testing

Figure 2. Methods to circumvent the BBB. Advantages and limitations of the different methods used to circumvent the BBB for drug delivery to the brain are compared.

pathogens to the brain.[5] However, as mentioned, the BBB also limits the entry of pharmaceutical agents, thereby making the brain disorders notoriously difficult to treat.[2] Moreover, the presence of efflux transporters, such as p-glycoprotein and multidrug resistant proteins, act to intercept or shuttle out lipophilic drugs, conferring a resistance of the brain to drug therapy.[6] In fact, the presence of the BBB is sometimes the sole reason for the clinical failure of even a highly potent therapeutic agent.[7]

APPROACHES TO CIRCUMVENTING THE BBB

There are three broad categories of methods used to circumvent the BBB for drug delivery: (A) novel drug design or drug modification for improved access through the BBB, (B) bypassing the BBB or using surgical intervention for delivery of drugs by an implantable device to the brain, and (C) use of chemical agents or other techniques to temporarily increase BBB permeability (Figure 2).

(A) Small molecule drugs have been developed for effective treatment of epilepsy, schizophrenia, chronic pain, and depression;[2] however, most small molecule drugs do not cross the BBB. In addition, large molecule therapeutics, such as antibodies and peptides which represent some of the most promising drugs currently in the clinical pipeline, do not cross the BBB at all.[8,9] Drug modification of these agents, such as using carrier molecules, is expensive and often does not result in significant concentrations at the target.[10] As well, the high intravenous doses of these drugs which are required to achieve substantial accumulation in the brain may lead to adverse effects in the peripheral organs.

(B) There are both noninvasive and invasive routes to bypassing the BBB. The noninvasive method of delivering drugs through the nasal epithelium has been investigated. Intranasal delivery has been effective for agents such as stem cells in preclinical models;[11,12] however, for drug delivery, this technique requires movement of the drug through the brain parenchyma and penetration into the region of interest. The reduced nasal epithelium in humans compared to rodents, accompanied by the slow drug diffusion through parenchyma, has resulted in low delivery efficiency in clinical studies.[13,14] Surgical intervention is an invasive method of bypassing the BBB for drug delivery. Intracerebral or intraventricular injection has been used for effective and direct delivery of therapeutic agents to the brain[15] or for placement of an implant for long-term targeted drug delivery.[16−18] However, surgical intervention is highly invasive and adds unnecessary risks to the patients. In addition, surgery in the brain always results in the damage to healthy tissue.

(C) The intracarotid administration of hyperosmotic solutions such as mannitol causes shrinking of the endothelial cells and simultaneous stretching of the tight junctions[19] to aid in drug delivery to the brain. Other chemicals such as vasodilators[20] and solvents[21,22] have also been used to increase BBB permeability, but these agents are toxic and can cause neuronal damage. In addition, all of these agents result in widespread permeabilization of the BBB, allowing potentially cytotoxic compounds access to the entire central nervous system. As an alternative approach, we consider focused ultrasound a better option for noninvasive, reversible, and targeted BBB disruption for enhanced drug delivery to the brain.

FOCUSED ULTRASOUND (FUS)

Technological advances with FUS have demonstrated the ability to use ultrasound in combination with preformed gas microbubbles to temporarily and reversibly increase BBB permeability, aiding in drug delivery to the brain.[23] FUS concentrates acoustic energy and deposits it in a small target volume in the brain with minimal or no consequences to the surrounding tissue.[23,24] The focal spot (area of highest energy) is contained within a small region at a targeted distance from the transducer surface. When electrical energy is applied, the piezoelectric material of the transducer converts that energy

dx.doi.org/10.1021/cn300191b | ACS Chem. Neurosci. 2013, 4, 519−526

into mechanical motion, thus generating ultrasound, which propagates through the skull and brain.

Early studies investigating the use of ultrasound to induce BBB disruption used the ultrasound focus to induce either elevated temperature or formation of gas bubbles (inertial cavitation) in the tissue to enhance the permeability of the blood vessels. This method found that BBB disruption was achieved but was almost always associated with damage.[25,26] In 2001, it was demonstrated that intravascular microbubbles used as contrast agents for diagnostic ultrasound imaging could concentrate the absorbed ultrasound energy in the blood vessels to create reproducible BBB disruption without observable long-term tissue damage.[23] During the sonication, the microbubbles expand and contract at the frequency of the ultrasound wave, a phenomenon known as acoustic cavitation (Figure 3). The oscillating microbubbles are thought to stretch

Figure 3. Acoustic cavitation and BBB disruption. Microbubbles (white) are injected intravenously at the onset of sonication. When the intravascular microbubbles enter the ultrasound field, they expand and contract at the frequency of the ultrasound. This leads to interaction with the endothelial cells and eventual BBB disruption.

the blood vessel walls to induce BBB disruption; however, the precise physical mechanisms are unclear. It has been postulated that oscillation of the microbubbles imparts stress on the endothelial cell wall[27] as observed by vessel wall displacement[28,29] and the detection of acoustic microstreaming.[30,31] Simulations have suggested that local temperature rise[32] may contribute to disruption even without an accompanying increase in tissue temperature. The mechanism of BBB disruption may vary with pressure amplitude, vessel diameter, and elasticity, among other variables.[27] Regardless, intravenous administration of microbubbles allows the BBB to be opened using a significantly reduced acoustic power, over 100 times less than that required to produce thermal damage in the tissue.[23,25]

Magnetic resonance imaging (MRI) has been the primary imaging modality used to target and monitor BBB disruption with FUS. MRI offers excellent soft tissue contrast, so it is optimal for identifying and targeting specific brain structures (Figure 4A). In addition, contrast-enhanced imaging can be used to confirm BBB opening (Figure 4B), since the contrast agent is too large to pass the intact BBB "outside of the ultrasound focus."

Following the initial study which combined low intensity ultrasound with microbubbles for BBB disruption,[23] there have been several studies aimed at optimizing the parameters for BBB disruption without associated tissue damage. Ultrasound frequencies used for transcranial BBB disruption in rodents range from 28 kHz[33] to 8 MHz,[28] although the clinically relevant range is likely between 0.2 and 1.5 MHz. The amount of pressure required for BBB disruption is greater for higher frequencies[34] and therefore needs to be adjusted for each experiment design. Pulse lengths ranging from a few microseconds to 100 ms can induce BBB disruption with no real benefit for longer pulse lengths.[23,35−37] Other parameters such as microbubble size and dose can affect the extent of BBB

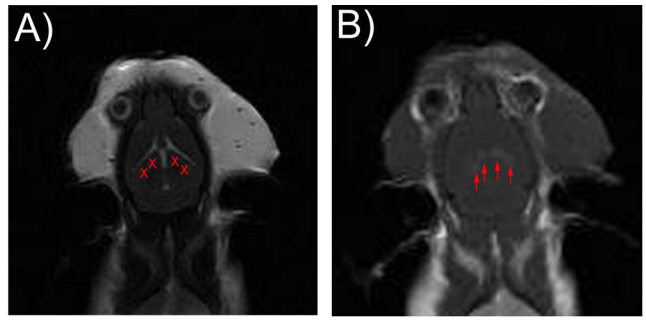


Figure 4. Targeting and monitoring of BBB disruption with MRI. (A) T2-weighted images (acquired on 7T Bruker Biospin 7030, Germany) were used for targeting. Four spots in the dorsal hippocampus were chosen, and each spot was marked with an x. (B) Following sonication, gadolium contrast agent (0.2 mL/kg; Omniscan, GE Healthcare, Milwaukee, WI) was injected in the tail vein and T1-weighted images were acquired. Areas of hyperintensity are indicative of BBB disruption. Four hyperintense spots corresponding to the targeted locations are clearly visible, indicating that the sonication was successful.

disruption.[38,39] Numerous efforts have been made to optimize BBB disruption, but perhaps the biggest contribution has come from development of a real-time acoustic controller.[40] Acoustic emissions are monitored and used to actively control the pressure amplitude of the ultrasound leading to a consistent BBB disruption.[40]

■ ADVANTAGES OF FUS FOR BBB DISRUPTION

FUS has several advantages which overcome the limitations of the other methods used to circumvent the BBB.

Noninvasive. Ultrasound energy is capable of transmitting through the skull and brain and therefore does not require surgical intervention.[23] As well, unlike other methods to disrupt the BBB, an intracarotid injection is not necessary. The microbubble contrast agent has a half-life in circulation of approximately 2 min and therefore can be administered intravenously and repeatedly.

Targeted. Osmotic and chemical disruption of the BBB is widespread so drugs are delivered throughout the brain tissue.[19] Similarly, using intranasal delivery methods, the drugs must move through the brain parenchyma to reach the target region. Conversely, FUS is able to target structures as small as the size of the focal spot or as large as required. For example, Thèvenot et al. delivered EGFP viral vector to the mouse striatum and hippocampus separately, demonstrating the ability of FUS to target specific structures.[41] On the contrary, Jordão et al.[42] chose four sonication locations in the cerebral cortex, essentially opening the BBB throughout the entire hemisphere. These studies provide a snapshot of the targeting capabilities of FUS for both small and large brain structures.

Transient. The BBB has been found to be reversible and is fully closed as early as 6 h postsonication and remains impenetrable up to 4 weeks later[24] as long as exposures are kept below levels inducing tissue damage. However, there are studies which show that the opening can last up to 24 h at similar exposure levels.[23,43] It is not currently clear what contributes to the differences between these studies. One caveat of FUS research to date is that most studies have been completed in healthy animals. Future studies are required to ensure that closure of the BBB is as efficient in models of neurodegenerative diseases as it is in the normal brain.

dx.doi.org/10.1021/cn300191b | ACS Chem. Neurosci. 2013, 4, 519−526

Safe. When appropriate ultrasound parameters are used in conjunction with microbubbles, there is no histological evidence of ischemia or apoptosis following BBB disruption.[23,24] The entry of blood components such as red blood cells and albumin are thought to be neurotoxic; however, studies have shown that the minor extravasations following BBB disruption are cleared by glial cells and do not adversely affect the neuronal population.[24,44] Cognitive and motor tests performed following BBB disruption at multiple points in the brain showed that FUS had no adverse effects on behavior.[45,46] However, use of inappropriate ultrasound parameters can result in tissue damage. The acoustic monitoring technique has improved the safety of BBB disruption.[40] Acoustic emissions are monitored and used to actively control the pressure amplitude of the ultrasound, leading to consistent, safe level of BBB disruption.

CELLULAR MECHANISMS OF FUS

Passive cavitation detectors have been used to show that FUS induced disruption of the BBB is due to an interaction of the microbubbles with the walls of the cerebral capillaries; however, the precise mechanisms of interaction are unclear. Several studies have indicated that there are different means of transport across the BBB following FUS, including transcellular and paracellular routes.

Transcellular passage of tracer molecules has been described using electron microscopy.[43,47,48] Sheikov and colleagues described cytoplasmic channels and a greater number of vesicles in the endothelial cells of the BBB post-FUS, suggesting increased pinocytosis/endocytosis. In a related study, high resolution microscopy combined with high-frame rate recordings demonstrates that microbubble oscillation in the ultrasound field causes deformation of endothelial cells.[49] Fluorescent dye uptake was measured and found to only occur in deformed cells, suggesting that oscillating microbubbles enforce pore formation in the cell membrane, leading to drug uptake.[49,50] Active uptake of drugs and tracer molecules has also been demonstrated. Gold particles and labeled IgG were delivered intravenously during sonication and were later identified within the endothelial cell caveolae using electron microscopy.[48] In support of the presence of an active transport mechanism, it was recently shown that FUS upregulates caveolin-1 leading to a peak in BBB permeability at 1 h postsonication.[51] Active transport of tracer molecules across the blood-tumor barrier (BTB) is also enhanced with FUS as demonstrated by src phosphorylation and increase in caveolin proteins in rat tumor models.[52,53] In vitro work has also suggested that plasmid DNA can be taken up by clathrin-mediated endocytosis, but this is yet to be shown in vivo.[54]

Paracellular transport of drugs across the BBB is hypothesized to occur via stretching the tight junctions, via disruption of the tight junctional proteins, or, in rare cases, by way of injury to the endothelial lining.[29] Electron microscopy has been used to visualize the widened tight junctions.[43] In addition, the transmembrane proteins which make up the tight junctions (occludin, claudin 1, and claudin 5) have been shown to be downregulated following BBB disruption.[43,55] Recent work by Jalili et al.[55] demonstrated that FUS disrupts tight junctional protein complexes and induces Akt signaling from neurons in the regions of BBB disruption up to 24 h postsonication. Akt has previously been implicated in neuronal survival and in hyperpermeability of the BBB.[56] Another potential mechanism contributing to the widening of the tight junctions is through the reorganization of gap junction proteins connexin 36 and 43.[57] Connexin 36 and 43 interact with the tight junction protein, zonula occludens, and their redistribution may lead to increased permeability through stretching the tight junctions. It is thought that entrance of macromolecules into the brain postsonication is via the paracellular route.

Recent work using two-photon microscopy has described three distinct patterns of dye leakage from the cerebral vasculature following BBB disruption.[36,58] The observation of diffuse dye leakage along the length of the vessel was postulated to be due to transcellular passage compared to intense focal leakage, postulated to be due to paracellular transport.[36] Controlling the range of applied ultrasound pressure could be used to induce the different type of leakage following BBB disruption. Managing the leakage types, indicative of BBB disruption, may further improve drug delivery using FUS.

Using two-photon microscopy, the potential role of support cells, such as astrocytes and microglia, in regulating BBB permeability and repair following FUS is being investigated.[59,60] Using EGFP Wistar rats, it was observed that the astrocyte endfeet detached from the vessel wall corresponding to the disruption of the BBB, suggesting that astrocytes may contribute to the increase in BBB permeability. Two-photon microscopy will be a valuable tool for further investigation of cellular mechanisms responsible for BBB disruption and drug delivery following FUS.

The recovery of the BBB functionality has been reported to be between 6 and 24 h for small tracer molecules such as gadolinium,[61,62] and by 12 h the levels of tight junctional proteins have been restored.[63] Contrast enhanced MRI has shown that once the BBB is closed following FUS, the barrier remains impenetrable up to 4 weeks later.[24] Regardless of the precise mechanism of passage across the BBB, it has been repeatedly observed that enhanced permeability of the BBB by FUS and microbubbles leads to greater drug accumulation in the brain.

FUS IS AN EFFECTIVE DRUG DELIVERY METHOD

Over the past decade, several agents which do not normally cross the BBB have been shown to accumulate in the brain following delivery with FUS. Most commonly, gadolinium-based contrast agents [~900 Da] are used to confirm BBB disruption in MRI guided treatments.[23,29] In other studies which were designed to evaluate the optimal ultrasound parameters for BBB disruption, histological tracers including Trypan and Evan's blue (~900 Da) and horseradish peroxidase (40 kDa) were delivered.[48,64] Using two-photon and other forms of fluorescent microscopy, tagged dextrans of various molecular weights serve as drug models.[58,65,66]

The first evidence that FUS could enhance immunotherapy in the brain was published in 2006 with the delivery of the powerful anti-breast-cancer agent, Herceptin, to the brain of normal mice.[67,68] A recent study showed that median survival times could be significantly increased by using FUS to deliver Herceptin in a rat model of breast metastasis to the brain.[69] Another recent approach used FUS to deliver targeted natural killer cells to breast tumors implanted in the brain.[70] Histological detection of apoptosis inducing factors suggests the cells remain bioactive after delivery through the BBB. Delivery of other chemotherapeutic agents including 1,3-bis(2-chloroethyl)-1-nitrosurea (BCNU),[71,72] epirubicin,[73] and doxorubicin[74,75] have all resulted in decreased tumor growth and improved animal survival.

dx.doi.org/10.1021/cn300191b | ACS Chem. Neurosci. 2013, 4, 519−526

There has also been interest in using FUS for treatment of Alzheimer's disease (AD). Initial studies aimed at addressing the safety of BBB opening in AD mice were necessary since the amyloid pathology associated with Alzheimer's disease is known to significantly impact BBB structure and function.[76] The results of these first studies demonstrated that BBB disruption using FUS was not significantly different in AD transgenic mice, compared to wild-type controls.[77] Furthermore, in a similar AD mouse model, it was found that the opening of the BBB was comparable in aged mice (12 months vs 26 months), despite the increased brittleness of the skull and altered vasculature characteristic of the older transgenic animals.[78] Raymond and colleagues[78] also delivered amyloid beta antibodies to the brain of the AD mice and found they colocalized with the amyloid plaques, demonstrating the feasibility of using FUS for AD immunotherapy. The first study to demonstrate that delivery of amyloid antibodies by FUS could reduce AD pathology showed a significant reduction in plaque size and number following a single treatment.[42] Efforts to understand the mechanism of antibody therapy in the AD brain following FUS are underway.[79,80]

Noninvasive gene therapy is also being explored using FUS. AAV-9 carrying green fluorescent protein was delivered to one focal point in either the striatum or the hippocampus.[41] Using FUS, reduced doses of the virus were administered intravenously, resulting in enhanced transgene expression in the brain but with minimal infection in the peripheral organs. Another group used FUS to demonstrate the feasibility of gene therapy by delivering brain-derived neurotrophic factor (pBDNF-EGFP-N1) loaded into microbubbles.[81] siRNA therapeutics for gene silencing have also been shown to be delivered to the brain using FUS.[82] Cholesterol-conjugated siRNA directed to the huntingtin protein was given intravenously and shown to significantly reduce huntingtin expression in the sonicated brain regions.

Stem cells have also been delivered to the brain, most likely through the widened tight junctions following FUS mediated BBB disruption.[83] Neural stem cells are approximately 7−10 μm in diameter, similar to the size of red blood cells, a few of which were reported to extravasate during BBB disruption; however, no adverse effects were observed.[83]

CLINICAL TRANSLATION OF FUS FOR BBB DISRUPTION

Single element transducers are effective for transmitting ultrasound through the relatively thin rodent skull[23,84] and even in nonhuman primates;[85] however, the thickness and variability of human skulls pose a significantly greater challenge. The design of clinical transducers are hemispherical and have a geometric focus which can be electronically steered in order to disrupt the BBB in regions off the midline.[86] The low frequency, hemispherical phased arrays reduce skull aberrations and distribute heat over the entire skull surface.[86]

The commercial prototype for clinical transcranial FUS (Exablate 4000, Insightec, Israel) has been used for BBB disruption in both swine[87] and nonhuman primate models.[46] In the primate model, BBB disruption was achieved in multiple locations throughout the brain. Animals showed no behavioral deficits over several weeks and performed well in cognitive tests.[46] This study has suggested that BBB disruption with FUS is ready for clinical testing in humans.

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Funding

Support for this work was provided by grants awarded by the National Institutes of Health (R01 EB003268) and the Canadian Institutes for Health Research (FRN 119312).

Notes

The authors declare no competing financial interest.

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