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Drug delivery across the blood-brain barrier using focused ultrasound

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Abstract

Introduction—The presence of the blood-brain barrier (BBB) is a significant impediment to the delivery of therapeutic agents to the brain for treatment of brain diseases. Focused ultrasound (FUS) has been developed as a non-invasive method for transiently increasing the permeability of the BBB to promote drug delivery to targeted regions of the brain.

Areas Covered—The present review briefly compares the methods used to promote drug delivery to the brain and describes the benefits and limitations of FUS technology. We summarize the experimental data which shows that FUS, combined with intravascular microbubbles, increases therapeutic agent delivery into the brain leading to significant reductions in pathology in preclinical models of disease. The potential for translation of this technology to the clinic is also discussed.

Expert Opinion—The introduction of MRI guidance and intravascular administration of microbubbles to FUS treatments permits the consistent, transient, and targeted opening of the BBB. The development of feedback systems and real-time monitoring techniques improve the safety of BBB opening. Successful clinical translation of FUS has the potential to revolutionize the treatment of brain disease resulting in effective, less-invasive treatments without the need for expensive drug development.

Keywords

Focused ultrasound; blood-brain barrier; drug delivery; neurodegenerative disease; brain tumor

3. General overview of the blood-brain barrier (BBB)

The blood-brain barrier (BBB) is a selective, highly-regulated barrier created by the endothelial cells in the cerebral vasculature [1]. It was first discovered after observing that systemic injection of dye results in the staining of all organs except for the brain [2]. Since, the BBB has been characterized as a complex structure which strictly regulates molecular transport into the brain.

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3.1 Cellular Structure and function

The structure of the BBB is characterized primarily by the presence of tight junctions between adjacent endothelial cells which allows the BBB to function as a physical barrier to the passage of molecules into the brain [3]. Tight junctions are created when membrane bound cell adhesion molecules such as claudins and occludins, form homodimers between adjacent cells. These protein networks prevent the paracellular passage of molecules and force molecules to take a transcellular route into the brain. However, the cerebral endothelial cells contain fewer transport vesicles than peripheral endothelial cells, thereby limiting transcellular molecular transport. Beyond the endothelial cells are the cells of the neurovascular unit which regulate and support the barrier. The neurovascular unit contains the basal lamina and a complex cellular system of astrocytes, pericytes, microglia and neurons that function together to support the BBB [3].

3.2 Barrier for Drug Delivery

The BBB has many roles which make it essential for proper brain function. Most importantly, the BBB functions to maintain a delicate homeostatic environment by regulating ion and neurotransmitter concentrations, and preventing the access of toxins, immune cells and pathogens from the peripheral circulatory system [1]. Moreover, the BBB regulates the transport of nutrients into the brain and assists with removing waste products. While these functions are necessary for maintaining health of the brain, when brain diseases develop, the BBB also prevents the access of therapeutic agents thereby making brain diseases notoriously difficult to treat [4]. It has been estimated that the BBB prevents the access of over 98% of potential therapeutics from passing into the brain [4]. As well, lipophillic and other small molecule drugs which are able to pass through the endothelial membrane are intercepted and shuttled out by the presence of efflux transporters. The BBB is the single most important factor limiting the treatment of brain diseases today. Recent studies have suggested that the financial burden of brain disease on society is double that of cancer [5].

4. Current Approaches to Drug Delivery in the Brain

Several methods have been developed to overcome the BBB and allow drugs into the brain. Surgical approaches are the most effective for direct drug delivery into the region of interest. Specific brain regions are targeted using stereotactic coordinates and needles or pumps are used to deliver drugs by direct injection. Although positive results have been seen for in clinical trials for -Parkinson’s and Alzheimer’s Diseases [6,7] the potential for damage to healthy tissue due to the needle track is great and add to the general risks associated with anaesthesia and surgery. Less invasive than surgical injection directly into the brain is injection into the cerebrospinal fluid. Intrathecal injection bypasses the BBB but drug concentrations at the target are unpredictable.

Other methods to induce temporary disruption of the BBB for drug delivery include use of chemical agents such as sugar alcohols, solvents, and vasodilators [5,8]. Mannitol, a hyperosmotic solution which shrinks the endothelial cells and stretches the tight junctions, has been often utilized to effectively promote drug delivery to the brain. However, mannitol,

and other chemical permeabilization agents, induce widespread BBB disruption exposing healthy brain tissue to the therapeutic molecules and other compounds from the circulatory system [9]. As well, mannitol has the potential for renal complications, especially after repeated exposure.

Novel methods have been developed to improve drug delivery to the brain by taking advantage of the natural receptors and anatomical features of the BBB. Drug modification to increase intracellular uptake by brain endothelial cells has shown to result in bypass of the BBB however often the agents do not reach therapeutic concentrations in the brain [10]. Alternatively, intranasal administration of drugs has shown to be effective for increasing therapeutic concentrations in the brain. Recently, using fluorescent tracers, it has been shown that intranasally delivered therapeutic agents may travel through the perivascular space to reach distant brain regions [11]. However, this method requires that the drug penetrate large brain regions which may be challenging in the human brain [12].

This review will discuss the development of focused ultrasound, a novel, non-invasive, targeted method for improving drug delivery to the brain.

5. Focused Ultrasound-Mediated BBB opening

The first reports that ultrasound could be used to permeate the BBB were published in the 1950's. The early studies showed that ultrasound could increase BBB permeability however it was accompanied by haemorrhage or thermal coagulation [13,14]. The mechanism was suggested to be both thermal and cavitation-mediated. Thermal-mechanisms were hypothesized since BBB permeability is observed in the regions surrounding a thermal lesion. Although successful using *in vitro* BBB models [15], investigation into the threshold for thermally-induced BBB opening *in vivo* indicated that thermal opening of the BBB is always associated with tissue damage [16]. Thus, while it is possible to use hyperthermia to induce BBB disruption, these approaches are currently unsafe.

High intensity focused ultrasound (HIFU) has been used to induce cavitation, the generation and collapse of bubbles within the tissue, and induce BBB opening without significant macroscopic elevation in brain temperature. In general, haemorrhage and tissue damage occurred more often as the pulse duration, pulse number and repetition frequency increased [17]. Although BBB opening was possible, the related bioeffects were unpredictable and varied extensively between studies [17,18].

The addition of preformed microbubble ultrasound contrast agent was found to reduce the acoustic pressure amplitude required for effective BBB opening, transforming the use of FUS in the brain [19]. Combining FUS and microbubbles produces consistent, reproducible and transient BBB opening without damage to the brain tissue [19]. Mechanistically, the microbubbles concentrate the ultrasound energy thereby reducing the required ultrasound power by more than 100 fold [20]. The microbubbles are important for reducing the amount of energy required to pass through the skull. The lower the energy requirements through the skull, the lower the potential for skull heating, thereby making transcranial treatments feasible and safer. When the circulating microbubbles pass through the ultrasound field, the microbubbles expand and contract, interacting with the blood vessel wall and leading to

increased permeability of the BBB. Using low pressure, increased BBB permeability can be achieved and side effects are restricted to a few extravasated red blood cells [19].

The use of magnetic resonance imaging (MRI) has been effective as a guide for targeting and as an evaluation of BBB opening. The excellent tissue contrast and ability for contrast-enhanced imaging to assess changes in BBB permeability have made MRI the primary imaging modality for FUS treatments (Figure 1).

In the past decade, reports from many different groups have demonstrated that different ultrasound parameters can be used to open the BBB. BBB opening has been achieved using frequencies ranging from 28kHz [21] to 8MHz [22]. The range that is relevant for clinical use is between 0.2MHz and 1.5MHz. due to the large focal spot size at low frequency and high pressure requirements at high frequency [23]. In addition to frequency, other ultrasound parameters including burst duration have been shown to positively correlate with BBB opening [24–26]. With respect to pulse repetition frequency, it has been suggested that adequate time is required to allow time for reperfusion of the microbubbles [27] however, changes in burst repetition frequency did not affect changes in BBB permeability [24]. Microbubble concentration and size have been shown to be positively correlated with greater BBB opening and potential for damage [28–32].

The development of a real-time, acoustic controller has reduced the variations of BBB opening and moved on step towards optimal BBB opening using FUS [33]. The feedback controller will be discussed further in the Safety and Treatment Monitoring.

5.1 Benefits and Limitations

The benefits of using FUS and microbubbles as a method for transient BBB opening over other methods for drug delivery to the brain are numerous. First, FUS can pass through the skull and therefore does not require invasive surgical methods. Using non-invasive procedures to replace surgery reduces the risks of general anaesthetic and surgery. The administration of microbubbles at the onset of FUS is performed intravenously thereby eliminating the need for invasive intracarotid injection associated with mannitol. Second, imaging methods can be used to target the ultrasound to the brain region of interest so that drug delivery and exposure to the peripheral blood products is limited to brain regions affected by disease. This is advantageous over the widespread BBB permeabilization achieved with pharamacological inhibitors and avoids damage to healthy brain tissue which occurs with surgical interventions. The size of the FUS-treatment area is correlated to the size of the focal spot of the transducer, with higher frequencies producing a sharper focal spot. Using higher frequencies, specific brain regions have been targeted for delivery of chemotherapeutics to a brain tumour or stem cells to the striatum or hippocampus [34,35]. However, FUS can also be used to target widespread pathology as in Alzheimer’s disease. In rodents, whole-hemispheres have been treated using lower frequency transducers and multiple sonications [36] and whole brains have been treated using unfocused ultrasound transducers [37]. Targeted FUS treatments have been shown to be possible at a variety of locations in preclinical models. Third, changes in BBB permeability following FUS treatment are transient and the BBB has been shown to functionally recover within 6 hours post-treatment. The literature has varied with respect to the time that the BBB remains open

following FUS [31], however it is clear that in studies when ultrasound parameters that don't result in damage are used, the BBB is closed in 6 hours and remains impenetrable for up to 4 weeks [19]. A limitation of research using FUS and microbubbles to open the BBB is that most of the FUS studies performed to date have been on healthy animals and thus further investigation is required in order to confirm that changes in BBB permeability occur on the same time course in models of disease.

5.2 Physical and Cellular Mechanisms

The precise physical and cellular mechanisms leading to transient opening of the BBB following treatment with FUS and microbubbles remain unclear. In general, the hypothesized mechanisms can be grouped into physical effects exerted on the blood vessel or cellular changes at the level of the BBB.

Using high intensity ultrasound, bubbles are formed in the tissue and undergo a violent collapse. The threshold for this inertial cavitation is variable and often leads to tissue damage and haemorrhage [17]. Since, the addition of microbubble contrast agent, low ultrasound powers have be used to induce cavitation, resulting in microbubble oscillation and growth [19]. This predictable oscillatory behaviour of microbubbles within an ultrasound field is known as 'stable' cavitation and is believed to be the cause of BBB opening. Microbubble oscillations generate mechanical stress when they interact with the vessel wall [38]. Using high speed microscopy, microbubble expansion and contraction has been shown to stretch the blood vessel wall with the contraction phase imparting the greatest stress on the vessel wall [39]. Oscillating microbubbles also generate circumferential and shear stresses, which contribute to the pressure on the microvessel [40]. The combination of these mechanical stresses may activate mechanosensitive ion channels in the endothelial cells leading to BBB opening. Similar to what occurs in the absence of microbubble contrast agent, if the pressure is too high, microbubbles collapse, causing high-velocity microjetting and extreme local temperature rise [40,41]. Analysis of the harmonic and ultraharmonic emissions suggest that bubble oscillation is associated with BBB opening and that broadband emission, indicative of microbubble collapse is correlated with red blood cell extravasation and tissue damage [24,33]. The impact of the vessel wall on microbubble behaviour was recently characterized and determined that the threshold for inertial cavitation and potential for damage depends on vessel size.

The physical stresses imparted on the vessels due to interactions between the microbubble and the ultrasound lead to cellular changes at the BBB. Electron microscopy was used to demonstrate that drugs and tracer molecules are able to traverse the BBB using both paracellular and transcellular routes after FUS treatment [42,43]. Labelled endogenous IgG was found inside vesicles of endothelial cells providing evidence that molecules pass transcellularly through the brain endothelium and accumulate in the brain. Further evidence in support of transcytosis as a method for drug delivery after FUS includes an increase in the cytoplasmic channels and vesicles. Caveolin, a protein integral to endocytosis, is upregulated following FUS treatment as demonstrated by immunohistochemistry and Western blot procedures [44,45].

In addition, physical stresses of FUS and microbubbles have lead to increases in the paracellular passage of molecules via opened tight junctions [42,43]. Reduced tight junction proteins have been detected using electron microscopy. In particular, significant reductions in occludin, claudin-5 and zonula occludens were observed at 1 and 2 hours post-FUS treatment. The levels of the proteins were returned to presonication levels by 4 hours confirming that the effects of FUS on the barrier are transient [43]. These data were consistent with the use of pharmacological inhibitors such as mannitol in which occludin, claudin 5 and zonula occludens were reduced alongside BBB opening. It is possible that FUS downregulates the expression of the proteins or that FUS simply alters protein organization causing a masking of the antigen [43]. In fact, a gap junction protein, Connexin 36, which binds to zonula occludens-1, has been shown to be downregulated following FUS [46]. The protein is responsible for communication between neurons and astrocytes and one hypothesis is that reorganization of the gap junctions are part of a response to changes in BBB permeability to help protect the brain [46].

The use of two-photon microscopy for characterization of BBB opening following FUS with microbubbles has provided new insights regarding the cellular mechanisms of BBB opening [47,48]. BBB permeability was further characterized into either ‘fast leakage’ and ‘slow leakage’ based on the temporal dynamics of the leakage of the fluorescent dye from the blood vessels [48]. Fast leakage was characterized as a rapid increase to peak intensity during FUS treatment with a decrease after FUS treatment was finished. Conversely, slow leakage began 5–15 min after the FUS treatment ended occurring along the length of a vessel instead of in a localized spot. The authors hypothesized that the two leakage types correlated to paracellular transport (fast leakage) and transcellular transport (slow leakage). If future studies could combine two-photon microscopy with acoustic information, the relationship between the microbubble behaviour and the leakage type could be elucidated.

6 Drug delivery and Therapeutic Effects

6.1 Drug Delivery in Rodent Models

FUS and microbubbles have been used to effectively deliver a variety of tracers and therapeutic agents across the BBB using rodent models. MRI is most commonly used as a method for targeting and evaluation of the treatment and therefore, delivery of paramagnetic agents have been widely studied. Gadolinium-based contrast agents (500–900 Da) are the most commonly delivered agents since they can be used to confirm the success of the FUS treatment and evaluate the size of the treated region [19]. The relative amount of enhancement observed after on a contrast-enhanced T1-weighted MR scan is proportional to the relative amount of BBB opening.

Trypan blue and Evan’s blue (~70kDa when bound to albumin *in vivo*), are commonly used to visualize the regions of BBB opening on histology. Other tracers used to confirm BBB opening after FUS include horseradish peroxidase [42,43], MION-47 [48], Dextran conjugated fluorescent molecules [47,48], lanthanum chloride and superparamagnetic iron oxide [50,51]

FUS and microbubble mediated opening of the BBB has applications in treatment of brain tumours. The use of widespread chemotherapeutic agents have been effective for treatment of peripheral tumours however the limited ability to penetrate the BBB reduces their effectiveness in the brain. Doxorubicin has been shown to reach therapeutic concentrations when delivered to the brain using FUS [35, 52]. Follow-up studies demonstrated that treatment of brain tumours using FUS and Doxorubicin decreases tumour burden and improves median survival times [53,54]. Herceptin, an antibody which targets the HER2/neu receptor overexpressed on ~30% of breast cancers, has been effective at treating the primary disease but, like Doxorubicin, is ineffective for treatment of metastases to the brain. FUS was shown to enhance the delivery of Herceptin [55] and when delivered by FUS, Herceptin significantly reduced tumour burden and improved survival times compared to Herceptin treatment alone [56]. Other chemotherapeutic agents including Methotrexate [57], 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU)[58] and epirubicin [59] have also been delivered using FUS and have been shown to have tumour-reducing effects in the brain. Other anti-cancer agents including natural killer cells that were targeted to HER2/neu were also able to be delivered to the brain using FUS to permeabilize the BBB [60]. Despite their large size, the immune cells were found to enter the brain at quantities sufficient for tumour reduction.

Outside of cancer applications, opening the BBB with FUS and microbubbles has proven to be an effective delivery method in models of neurodegenerative disease. As a potential treatment for Huntington's disease, FUS was used to deliver, siRNA targeted to the mutant Huntingtin protein to the striatum of rats [61]. The siRNA delivery was effective, leading to an ~30% reduction of Huntingtin protein. This level of knockdown has been suggested to be sufficient for reducing symptoms associated with the disease. In a mouse model of Alzheimer's disease, the extent of BBB opening was determined to be similar in Alzheimer's transgenic mice compared to wildtype controls [47,62]. Delivery of amyloid antibodies using FUS [36,47] were found to significantly reduce plaque pathology after 4 days [36]. FUS-mediated delivery of neurotrophic factors including brain-derived neurotrophic factor (BDNF)[63], neurturin [63] and glial-derived neurotrophic factor (GDNF)[64] has been shown to result in levels high enough to induce neuroprotection and survival. Activation of downstream pro-survival pathways was observed following delivery of BDNF *in vivo* [63]. These therapies are important for treatment of neurodegenerative disease such as Alzheimer's and Parkinson's diseases in which growth factor levels are reduced. Another important therapeutic option currently being investigated is the use of neural stem cells as replacement cells in diseases characterized by neuronal loss. Even with their large size, neural stem cells were capable of crossing the BBB following FUS treatment [34]. Embryonic stem cells were delivered to the striatum and the hippocampus independently and were shown to differentiate after entry into the brain.

Finally, delivery vehicles for therapeutic agents including nanoparticles, viral vectors and microbubbles themselves, have gained access to the brain following FUS [63,65–68]. have been delivered to targeted areas of the brain and have a wide range of applications for treatment of brain diseases.

6.2 BBB Opening and Drug Delivery in Non-human Primates

FUS and microbubble mediated BBB opening has been performed in non-human primates. In studies completed by two different groups, BBB opening was achieved using FUS in the presence of commercially available and custom prepared microbubbles [68–70]. In one study, the non-human primates were sonicated using a commercially available hemispherical transducer array [71]. The design of the clinical transducer reduces the aberrations in the transmitted ultrasound waves created by the variations in skull thickness and density [72]. Non-human primates were treated in multiple locations at different treatment times [71] and were found to perform equally well before and after the treatments. Similar evidence was observed using a single element transducer for ultrasound delivery [69,70]. This evidence strongly supports the safety of FUS for BBB opening in the brain.

6.3 Therapeutic Effects of Ultrasound Alone

Several studies have been designed to characterize the response of the brain to FUS treatment and therefore have been performed in the absence of exogenous drug delivery. Recent studies suggest that BBB opening due to FUS and microbubbles may have therapeutic effects in the brain, even in the absence of drug delivery. Using a mouse model of Alzheimer's disease, FUS-mediated BBB opening was able to reduce plaque burden even in the absence of amyloid antibodies [73]. Two potential mechanisms were investigated to explain the phenomenon. First, the level of endogenous immunoglobulins (IgG and IgM) were significantly increased in the FUS-treated cortex suggesting that immunoglobulins can increase clearance of amyloid. Second, activation of astrocytes and microglia were detected in FUS-treated regions, suggesting that activated glia may internalize the amyloid and promoting clearance. Follow-up studies relating BBB opening by FUS and microbubbles to changes spatial memory behaviour are currently being completed and show promising results. This new data would support the finding that increased neurogenesis was observed in mice receiving FUS and microbubble treatment [74]. Non-transgenic mice were administered bromodeoxyuridine (BrdU) a thymidine analog which incorporates into dividing DNA, following FUS treatment. In the neurogenic regions of the hippocampus, a significant number of new neurons were observed suggesting that FUS increases neurogenesis [74]. These data correlate to previous published work indicating that FUS activates the pro-survival Akt signalling pathway [75]. Furthermore, it has been suggested that FUS without microbubbles, can increase the expression of BDNF, a neurotrophic factor which supports cell survival and differentiation [63,76]. FUS has been shown to induce neuronal activation when applied in the absence of microbubbles [76–79]. Together these data suggest that BBB opening by FUS and microbubbles may have beneficial effects in the brain in addition to its role as an effective method for drug delivery.

7 Clinical Translation of FUS Technology

Translation of FUS from rodents to the clinic presents challenges due to differences in skull shape and thickness. Clinical application of FUS will require the use of a hemispherical phased array transducer which reduces the potential for skull heating by distributing the ultrasound energy over the entire skull surface. The hemispherical arrays have a geometric focus that can be electronically steered by controlling the phase of the radiofrequency signal

driving the elements in order to open the BBB in regions away from the midline [72]. Applying the ultrasound at low frequency reduces the ultrasound wave distortion induced by the skull [72]. The commercially available clinical prototype devices are available at two frequencies, 220kHz and 650kHz (Exablate 4000, Insightec, Israel). The lower frequency device has been used for effective BBB opening in nonhuman primates [71]. Using the clinical device, multiple brain regions have been targeted and sonciated in a non-human primate model with no adverse effects on behavior. These studies have demonstrated that FUS, as a method for BBB opening, is ready for clinical testing in humans.

8 Safety and Treatment Monitoring

MRI has served as the primary method for evaluating BBB opening and the effectiveness of the FUS treatment [19] however MRI is limited because it can only be performed post-treatment. In an effort to provide real-time monitoring of the FUS treatment, several groups have developed methods which detect and monitor the acoustic emissions from the microbubbles [80–82]. In particular, the development of a real-time, acoustic feedback controller program has changed FUS treatments by enabling the active monitoring, and control of the BBB opening [33]. During the sonications, the controller increases the applied ultrasound pressure in a step-wise fashion with each transmitted pulse. A hydrophone, positioned to monitor the focal spot, receives the spectral information after each pulse. The spectral information is analyzed and when the presence of ultraharmonic emissions are detected, the algorithm reduces the pressure to a user-defined percentage. In rodent models, a reduction of the pressure to 50% of the peak pressure reached has been shown to result in BBB opening but without the presence of red blood cell extravasation or other signs of associated damage [33]. The controller can be combined with a novel multi-receiver method that allows microbubble localization with super resolution and perhaps control the activity at the level of a single bubble [83]. The implementation and testing of a controller system into a clinical array would allow the operator to monitor and control the FUS treatment thereby improving the safety of clinical FUS treatments.

9 Future Directions

Despite the significant advances in FUS over the last decade, there are still hurdles for this promising technology to overcome before it can be widely used in the clinic. Before repeated applications of FUS can be considered a viable technique to treat chronic diseases, further quantified research into monitoring drug concentrations in the brain will be necessary. Quantifying delivered drug concentrations will ensure therapeutic concentrations are achieved in the brain and prevent toxicity associated with high concentrations in the periphery. These questions may be able to be addressed by the addition of tracer agents which can be monitored by MRI but the suitability of these agents needs to be addressed in future preclinical studies. Furthermore, the intravenous drug concentrations required to achieve therapeutic dose in the brain after FUS-treatment may need to be adjusted from the dosages currently used clinically. It will be important to closely monitor the potential peripheral toxicity of these agents as dosages are altered.

The incidence of brain diseases which include cancer, vascular diseases, neurodegenerative diseases, psychiatric illnesses and others, are on the rise. Globally, brain diseases serve as a growing source of emotional, practical and financial burden as the race to find effective treatments continue. While there is much work to be done to move FUS-mediated BBB opening to widespread clinical use, the studies completed thus far indicate that the technology represents a promising strategy that will be integral to the future treatment of brain disease.

10 Expert Opinion

There is considerable evidence demonstrating the efficacy of FUS as a method for drug delivery into the brain. Furthermore, using several different rodent models of disease, it has been demonstrated that FUS results in accumulation of therapeutic concentrations of drugs at the target region leading to predicted bioeffects such as reductions in tumour size or Alzheimer's plaque pathology [36,53,54]. However, the acceptance of FUS as an alternative method for drug delivery has been slow and we believe this is due to several factors which are currently being addressed in the literature. In particular, we believe it is important to address the effects of FUS on a BBB compromised by pathology and to demonstrate using non-human primate models that the promising data reported in rodents can be achieved in a more-clinically relevant model.

For many decades, it has been known that the presence of a functioning barrier is necessary for maintaining homeostasis required for proper function of neuronal circuitry [1,3]. Adding to this, the breakdown of the BBB has been hypothesized to precipitate diseases such as Multiple Sclerosis and Alzheimer's disease [2]. Together, we understand that these data warrant caution when proposing to open the BBB for therapeutic purposes. Recent work has begun to address these concerns as more studies are being aimed at investigating the effects of BBB opening on the brain in the absence of drug delivery [19,69–71,73,75]. In general, these studies have collectively been unable to demonstrate any adverse side effect associated with transient BBB opening in the healthy brain. In some cases, opening of the BBB in rodent models of disease may actually lead to improvements in pathology and behaviour, even in the absence of exogenous drug delivery [73,74]. FUS is advantageous over other methods that transiently open the BBB since it can be targeted to a small focal volume. When employed as a treatment strategy, this means that only areas of the brain that exhibit pathology (tumours, plaques, inclusions and other pathology) would be exposed to BBB opening and the remaining healthy brain regions will not receive the therapeutic agent.

The differences between the rodent and primate brain have led to problems with clinical translation of other treatments however recent studies performing FUS-mediated BBB opening in non-human primates suggest this isn't the case with FUS. Studies completed by groups from Harvard and Columbia show data from non-human primates who were sonicated repeatedly at different locations in the brain [69,71]. The studies used different transducers to deliver the ultrasound, including the clinically available transducer array (Exablate 4000; Insightec, Haifa, Israel), and show no evidence of histological or functional damage after FUS treatments. These findings from non-human primates, combined with data gathered from rodent models, suggests that the transient breakdown of the BBB with FUS

does not lead to deleterious effects in brain tissue. The mounting evidence in support of the safety of transient, localized BBB opening may lead to an adjustment in conventional thought whereby in brain disease, the BBB is no longer thought of as a protective barrier but as a temporary obstacle to overcome for effective treatment.

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Article Highlights

- Focused ultrasound (FUS) combined with microbubbles increases the permeability of the blood-brain barrier (BBB) and improves drug delivery to the brain, leading to reduced pathology and increased survival in preclinical models of disease.
- FUS-mediated increases in BBB permeability have been shown to have beneficial effects on the brain even in absence of exogenous drug delivery.
- Feedback systems and real-time monitoring techniques have been developed which improve the safety of BBB opening.
- The technology required for effective clinical translation of FUS for drug delivery is currently available.

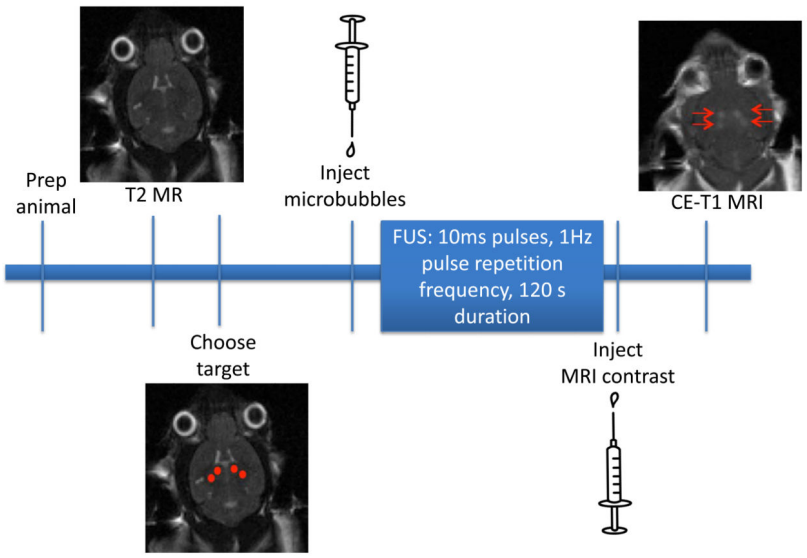

Figure 1.

Timeline for FUS experiments. Animals are prepared for FUS treatment by using chemical depilatory to remove the hair from the head and by inserting a catheter into the tail vein. A T2-MR image is acquired and the target locations for sonication are chosen (denoted by red dots). Microbubble contrast agent is diluted and injected immediately prior to the onset of sonication. FUS is applied using standard parameters (10ms pulses, 1 Hz pulse repetition frequency, 120s total duration). Following FUS, MRI contrast agent is injected and a contrast enhanced (CE)-T1 weighted MR images is acquired. The regions of hyperintensity correspond to areas where contrast agent move from the vasculature into the brain parenchyma and is used to confirm effective BBB opening with FUS.

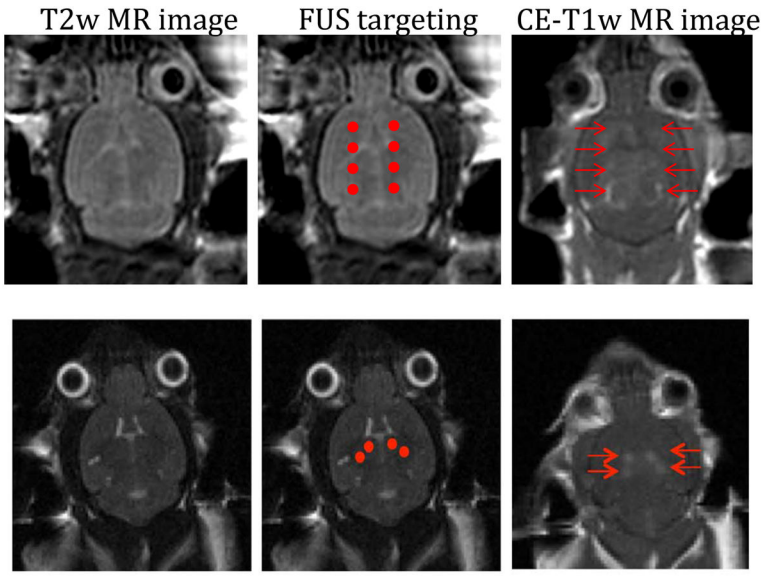

Figure 2.

MRI has been the primary imaging modality for targeting and evaluating FUS treatments. Top panel – MR images from a mouse brain were taken using a 3.0T MR scanner (GE Healthcare). T2 weighted images were used to choose 4 sonication points in each hemisphere (red dots). Contrast enhanced T1 weighted images were used to confirm BBB opening had occurred in the target locations (red arrows).

Bottom panel – MR images from a mouse brain were taken using a 7.0T MR scanner (Bruker). T2 weighted images were used to target the bilateral hippocampus using 2 sonciations in each hemisphere (red dots). T1 weighted images were used to confirm BBB opening had occurred on target (red arrows).

Table 1

Summary of the advantages and disadvantages of current methods used to circumvent the BBB for drug delivery to the brain

Strategy	Advantages	Disadvantages
Intracranial injection	• Delivery to targeted brain regions • Achieve therapeutic dose	• Invasive • Damage to healthy tissue
Drug Modification	• Option for personalized medicine • Noninvasive	• Difficult to achieve therapeutic concentration *in vivo* • Expensive and difficult to develop • Non-targeted
Intranasal Delivery	• Successful in pre-clinical studies	• Drugs must travel great distance in the brain • Difficult to achieve therapeutic concentrations *in vivo*
Global BBB disruption	• Transient opening • Can achieve therapeutic concentrations	• Non-targeted – entire BBB is disrupted
Focused Ultrasound	• Targeted to sub millimeter regions in the brain • Non-invasive • Therapeutic concentrations	• Only relatively short term studies have been performed.