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Neuroinflammation associated with ultrasound-mediated permeabilization of the blood-brain barrier

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Abstract

The blood-brain barrier (BBB) continues to represent one of the most significant challenges for successful drug-based treatments of neurological disease. Mechanical modulation of the BBB using focused ultrasound and microbubbles has shown considerable promise in enhancing therapeutic delivery to the brain, but questions remain regarding possible long-term effects of such forced disruption. This review examines the available evidence for inflammation associated with ultrasound-induced BBB disruption, and potential strategies for managing such inflammatory effects to improve both the efficacy and safety of therapeutic ultrasound in neurological applications.

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Author Contributions

OJ, AT and ES conceived the outline and directions for the literature review. OJ, AT wrote the early drafts of the manuscript and participated in the revisions of the subsequent drafts. ES coordinated the drafting process. SB provided advice and directions for flow and structure specifically in neuroinflammation section. MD, JF and MF provided feedback and guidance in later drafts of the manuscript.

Declaration of interests

Authors have no conflicts of interest to report.

Keywords

Blood-brain barrier; neurovasculature; focused ultrasound; microbubbles; cavitation; sterile inflammation; immunomodulation; immunoprivilege; neurological disease

Challenges for treatment of central nervous system (CNS) diseases

A significant proportion of the global population is diagnosed annually with some form of neurological disorder or disease – 16.5% of global deaths can be attributed to CNS diseases [1]. There have consequently been many efforts to develop effective CNS-acting compounds and biomolecules. Unfortunately, despite being one of the more heavily funded areas of research in the pharmaceutical industry, CNS drug discovery and development is associated with a low rate of return. Although there is an abundance of promising *in vivo* animal data from pharmacokinetic and pharmacodynamic studies, very few drug candidates show comparable efficacy in human trials [2,3]. Amongst the reasons for this are key differences in anatomy and physiology between humans and animal models including of the blood-brain barrier (BBB) [4].

Conventional methods of structural modification for small compounds have produced only modest improvements in terms of BBB penetration [5]. Consequently, in recent years, there has been increased interest in drug delivery methods to the brain based on local permeabilization of the BBB using focused ultrasound (FUS), especially in combination with microbubbles (MB). These methods have shown considerable promise, with several first-in-human clinical trials reporting successful outcomes[i], [ii], [iii] [6–8]. There are, however, important safety concerns relating to mechanical disruption of the BBB, specifically in relation to the metabolic and physiological pathways required for brain homeostasis. If the permeability of the BBB is modulated to increase drug extravasation, it is imperative to understand the potential consequences of that disruption, especially in neurological conditions in which BBB may already be compromised.

This paper aims to provide an overview of the evidence for ultrasound induced neuroinflammation, its implications, and strategies by which adverse effects could potentially be mitigated to maximize the benefit-risk ratio in clinical applications.

A brief overview of the blood-brain barrier

The BBB provides both a physical and a physiological barrier between the brain parenchyma and the bloodstream (Figure 1). It is composed primarily of microvascular endothelial cells supported by pericytes and astrocytic foot processes [9]. The BBB prevent entrance of exogenous toxins and agents from the bloodstream into the brain parenchyma and maintains separation between the CNS and the peripheral nervous system (PNS). Given the BBB's ability to selectively determine the passage of biomolecules and chemicals, its

[i] https://clinicaltrials.gov/ct2/show/NCT03321487
[ii] https://clinicaltrials.gov/ct2/show/NCT02986932
[iii] https://clinicaltrials.gov/ct2/show/NCT03608553

role in homeostasis, in multiple diseases, and in accurate evaluation of drug efficacy, are topics of great interest for clinicians and researchers.

Potential side effects of BBB disruption

There is a growing body of data demonstrating an important role for the BBB in mediating CNS diseases [10–13]. In developing drug delivery methodologies that disrupt the BBB, it is therefore important to consider the downstream effects of modulating the BBB (Figure 1). For example, BBB breakdown is known to coincide with peripheral immune cell infiltration and the inflammation of the brain parenchyma in diseases such as multiple sclerosis (MS) [14,15]. There is also some evidence of BBB involvement in the progression of other neurological diseases, such as lysosomal storage disorders [16–19]. For intensively studied neurological diseases such as Parkinson's (PD) and Alzheimer's disease (AD), there is now experimental evidence showing that the BBB may play an active role early on in their etiology [20–22]. In PD, accumulation of α-synuclein has been shown to be the dominant pathophysiology that leads to clinical manifestations observed in patients. While there have been previous reports of neurovascular impairment in PD patients, recent data have shown that with α-synuclein overexpression in mice, BBB integrity is also compromised [23]. In AD research, the accumulation of amyloid-β plaques and neurofibrillary tangles has been a central theme over the past few decades. In recent years, however, there has been increased interest in the effects of neurovascular factors as well [24]. For example, it has been proposed that a compromised BBB could allow passage of exogenous toxins and agents into the brain, leading to inflammatory responses that could cause plaque and tangle formation as a byproduct [25]. There is also evidence of a correlation between BBB degradation in AD tissue and bacterial and viral infiltration leading to an innate immune response cascade [26][27,28]. At the time of writing, these represent areas of considerable uncertainty and debate. For example, it has yet to be established whether BBB dysfunction is a causative agent in the disease processes, or a symptom of disease progression. These questions, nevertheless, have important implications for drug discovery, design, and development, as well as for preclinical *in vivo* drug evaluation. Leukocytic infiltration through the BBB is known to drive the pathophysiology in neuroimmune diseases [29], while non-specific transcytosis and tight junction dysregulation are upregulated in response to changes in the microenvironment surrounding neurons, e.g., during stroke [30]. The emerging use of alternative drug delivery techniques that modify the BBB integrity thus has to be balanced against the fact that many of the patients being treated may already have neurovascular complications and/or clinical symptoms that are driven by BBB dysfunction, as much as by neuronal dysregulation [11]. It is therefore critical to investigate the mechanism(s) behind BBB opening via ultrasound-mediated cavitation and the consequences of this manipulation, especially for the treatment of non-terminal diseases, for which patients may receive multiple treatments over several years.

Ultrasound and microbubble-mediated BBB opening

Initial studies.

FUS was first used therapeutically for tissue ablation. In this type of procedure, a large, single element, spherically focused transducer, operating at a center frequency between 0.5 and 10 MHz generates a region of sufficient intensity to cause tissue denaturation. Typically, the focal region of a FUS transducer is ~16 mm^3 which enables good spatial control of energy deposition. FUS can rapidly destroy tissue via a range of both mechanical and thermal effects. A common side effect of high intensity FUS is cavitation, i.e. the formation and subsequent oscillation of bubbles as a result of the changes in tissue temperature and pressure. The presence of these bubbles can be beneficial, for example accelerating the rate of heating and promoting mechanical erosion [31,32]. Cavitation is, however, a stochastic process and it was found that similar benefits could be achieved at much lower ultrasound intensities by injecting a suspension of pre-existing MB into the target tissue. This was particularly important in early, preclinical studies of BBB opening using FUS to mitigate the risk of collateral damage [33]. MB-mediated BBB opening was reported as a possible alternative drug delivery technique as well as a theranostic some two decades ago, when FUS was used with contrast agents and magnetic resonance imaging (MRI) to open and detect BBB opening in rabbits [34]. Subsequent studies in mice, rats, and rabbits, focused on the observed bioeffects, which included: vascular wall damage, ischemia and tissue necrosis [35]. The findings suggested that limiting parameters such as the acoustic pressure amplitude and pulse duration, would be critical in producing therapeutic effects with minimal adverse reactions [36,37]. There have also been multiple follow-up studies investigating the mechanism behind BBB opening [38]. Technical details regarding the physics of ultrasound and microbubbles can be found in Box 1.

Therapeutic applications.

Over the last two decades, the therapeutic potential of FUS+MB has been explored for a range of neurological conditions (excluding cancer) in pre-clinical models including the delivery of quercetin-modified sulfur nanoparticles to minimize endoplasmic reticulum (ER) stress in AD [39], BDNF retrovirus also for treatment of AD [40], curcumin and neurotrophic factors for treatment of PD [41,42] and to increase laronidase uptake as part of enzyme replacement therapy (ERT) in an animal model of mucopolysaccharidosis type I disease [43]. In clinical trials, FUS+MB with MRI guidance have been shown to enable localized BBB opening in amyotrophic lateral sclerosis (ALS) [6][i], AD [ii], and PD [iii] patients. Several studies, however, have highlighted potential risks associated with FUS+MB. These include neuroinflammation, which is discussed in more detail in the next section.

Ultrasound-induced neuroinflammation

Identification of sterile inflammation as a possible bioeffect of FUS+MB for BBB opening.

At the low frequencies (<1 MHz) required for efficient transmission of ultrasound through the skull, the probability of inertially driven bubble collapse is higher due to the prolonged rarefactional period. In vitro and modeling studies suggest that this can lead to significant

and permanent biological damage in the local tissue [44,45]. In addition, studies in rats from recent years have indicated that FUS+MB can induce sterile inflammation [46], a possibility that requires more extensive and rigorous investigation. In some applications, stimulation of an immune response may be beneficial, e.g. it has been suggested that FUS+MB may contribute to killer T cell activation and infiltration in tumors [43,47]. Similar approaches have been suggested for brain-specific tumors such as glioblastoma multiforme (GBM), an aggressive brain cancer with very poor prognosis [48].

Since the identification of FUS as a promising alternative delivery technique for CNS therapeutics, there has been extensive assessment of its safety. Table S1 provides a summary of selected studies using FUS+MB for BBB opening. Prior to 2017, a primary focus of the research on FUS for BBB opening was identifying acoustic parameters that minimize visible red blood cell (RBC) extravasation, as assessed by histological analyses in mice, rats and rabbits [36,49–54]. In recent years, studies, primarily in rats, have begun to address FUS-induced CNS inflammation in more detail, over time periods between 24h and 6 weeks post-ultrasound treatment and there have been several reviews and discussions of sterile inflammation as a response to BBB opening [55]. An area that requires further investigation, however, is the relationship between inflammation and the acoustic exposure parameters. There has been considerable investigation of how the selection of acoustic parameters affects the degree of BBB permeabilisation and how this relates to extravasation of differently sized molecules [51,56], but it remains to be examined whether there is a corresponding modulation of sterile inflammatory effects.

Mechanisms.

FUS+MB exposure has been shown to permeabilize the blood brain barrier through the disruption of tight junction protein complexes between endothelial cells – thought to be facilitated by oscillating MB along the endothelial surfaces [57,58]. Localized disruption allows blood-borne components such as circulating therapeutics or albumin to diffuse into the brain parenchyma. In addition to the formation of paracellular holes, neurovascular units may also be stimulated by the oscillating MBs. In rodent studies, this has been shown to stimulate a neuroinflammatory cascade, which upregulates the expression of chemokines, cytokines, and other relevant trophic factors [59–61] (Figure 2).

Several studies have suggested that permanent tissue damage is avoidable when the appropriate ultrasound settings and MB dose are used (Table S1). In view of these findings, subject-specific, pre-operative planning should be considered as a possible path to reduce tissue damage. In addition, active monitoring of MB response allows potential real-time feedback and control of the treatment by modifying the peak negative pressure and/or pulsing regime of the FUS, as exemplified in a recent preclinical study in non-human primates [62]. However, even when using the minimum acoustic settings to cause BBB permeabilization, it is conceivable that a sterile inflammatory response can still occur, although this requires further investigation. Studies in rats have shown that sterile inflammation following FUS-mediated BBB permeabilization is mediated through the NFkB pathway, with evidence of endothelial activation (high ICAM-1 expression) and a cytokine cascade including production of tumor necrosis factor, a potent inflammatory

cytokine, elevated even at 24 hours post sonication [63]. There have been studies looking at providing prophylactic treatment (i.e., anti-integrin α4β1/VLA-4) to mitigate possible immune infiltration or responses [64–66], but this has not been investigated specifically in the context of FUS+MB treatment for CNS diseases.

In recent years, significant efforts have been made to understand the mechanisms underpinning observable bioeffects following BBB opening at the cellular and molecular levels [67–69]. In particular, there have been several studies investigating specific immunomodulatory pathways [46,60,61,63,70] in microglia and astrocytes. Interestingly, there has been less investigation of the role played by endothelial cells and pericytes in potentially inducing the inflammatory cascade post-FUS+MB treatment. This is despite evidence suggesting that these cells and their interactions are critical to the process [71–76]. Better understanding of the initial physiological responses produced by endothelial cells and pericytes (with and without astrocyte and microglia activity) will be critical in assessing the cell-type specific effects of FUS+MB as well as the cell-cell crosstalk that ultimately generates tissue-level neuroinflammation.

It has also been shown that in rats, an innate immune response can be activated for up to 6 days after FUS+MB exposure, as evidenced by infiltration of CD68+ monocytes/macrophages [46]. Infiltration of the CNS by peripheral monocytes/macrophages is a hallmark of tissue damage that cannot be managed through microglial activation alone, and can be indicative of impending fibrosis [77], and potentially long-term implications. Even in cases where BBB integrity is restored within 24 hours post-sonication, the neuroinflammatory response does not always subside [70,78]. Additionally, FUS+MB exposure has been shown to reduce P-glycoprotein (Pgp) (encoded by the *ABCB1* gene) expression; this may allow for increased retention of therapeutics in the parenchyma, which could have immediate therapeutic benefits, but the downstream physiological effects should be further investigated, as Pgp expression and regulation are closely associated with pro-inflammatory and anti-inflammatory cytokine expression and release. [79,80]. Additionally, while there have been preliminary studies showing that FUS does not necessarily lead to tight junction complex damage [81], it has yet to be seen how non-homeostatic changes to the microenvironment may induce or facilitate biological changes in the integrity of the tight junctions or cellular membranes.

Clinical studies of the neuroinflammatory effects from FUS+MB treatment.

Table 1 presents an overview of recent clinical trials using FUS+MB in a range of CNS conditions, together with details of any inflammatory (or anti-inflammatory) pathways, where these are explicitly mentioned. In non-neurological conditions, FUS+MB exposure has been shown to stimulate immune responses that may be beneficial e.g. in metastatic cancer; or in other cases, to directly induce anti-inflammatory effects at the target site [82–85]. Recent studies have also shown that immunomodulation can be successfully used to treat GBM; and that FUS+MB can be effective in inducing targeted immune effects and to deliver immunotherapeutics with promising results [86,87]. It has yet to be determined, however, whether an immunomodulatory approach is appropriate for treatment of non-oncological CNS diseases [88–94]. While most current clinical trials report no

significant inflammation post-FUS+MB treatment (Table 1), the evaluation of potential neuroinflammation in many of these studies is limited, lacking for instance molecular biomarker data in regard to cytokine levels in the cerebrospinal fluid (CSF) or tissue biopsies.

Strategies for mitigating sterile inflammation in ultrasound-mediated therapy

As mentioned earlier, the use of FUS+MB has shown promising results for treatment of glioblastoma [95–97]. For as long as uncertainties remain over its long-term safety, however, the case for using FUS+MB in non-terminal CNS conditions is less clear [98]. There have been several studies looking at the immediate and short-term consequences of FUS treatment in humans, but these studies have focused primarily on functional measures designed to observe whether there were rises in biomarkers of concern [99,100]. To the best of the authors' knowledge, long-term follow up studies of the treated patients are still lacking. Such studies are inevitably difficult to perform, due to the complexity of neurological diseases and desegregation of confounding factors that may influence interpretation of clinical data. Examining long-term, post-procedure effects in animal models could provide one step towards addressing these complex issues.

There are already data showing that ultrasound-induced BBB disruption can induce inflammatory responses even at low acoustic intensities [46,61,101,102] and ultimately, the clinical applicability of FUS+MB is dependent upon understanding the underpinning mechanisms and the immediate, as well as long-term effects, of both single and multiple FUS+MB treatments. For example, it is critical to determine whether repeated treatment can produce adverse effects unrelated to the natural history of the neurological disease being treated. This is particularly important when identifying treatments for genetic and hereditary disorders in which many in the diagnosed population are pediatric patients.

Non-mechanical modulation of the BBB has also been shown to induce neuroinflammatory effects, indicating these are not specific to FUS+MB. For example, the use of d-mannitol, an osmotic agent that has been widely used for modulating intracranial pressure, has been reported to increase pro-inflammatory cytokines [103]. The opening of adjacent endothelial cells can induce a response from both astrocytes and microglial cells [60], such as a cascade of chemokines that encourage homing and chemotaxis of peripheral immune cells that are circulating in the neurovasculature, especially near the meninges [98]. A potential advantage of FUS+MB over d-mannitol is that the effects of FUS+MB can be much more easily localized to specific regions of the brain and their corresponding vasculature.

A key consideration for the use of FUS+MB in neurological disorders is the degree to which adverse reactions post-procedure can present and whether there are pre- or post-operative measures to minimize such effects. Some studies have shown that corrective measures can be taken post-treatment to inhibit an immune response using drugs such as dexamethasone [104]. In a similar manner, another group recently reported that the type of anesthetic used prior to FUS+MB disruption of the BBB can influence gene expression in the brain [105].

Such differences may not have immediate implications post-procedure but are likely to be critical in understanding how cells respond long after the initial acute disruption of the BBB.

Concluding remarks and future perspectives

This review has sought to examine the current literature on the role of the BBB in mediating sterile inflammation following exposure to FUS+MB. The number of studies that have evaluated sterile inflammation associated with FUS+MB, either *in vitro* or *in vivo*, is relatively small; and while the technology shows great promise, there is a need to accelerate our understanding of the downstream physiological responses (Figure 2). This need is becoming increasingly pressing as the range of applications for FUS+MB mediated BBB permeabilization increases and is extended into non-terminal conditions. Further work is needed to elucidate the pathways associated with such reactive inflammatory responses when the BBB is disrupted (see Outstanding Questions). Addressing this knowledge gap will hopefully encourage further discourse on potential improvements to FUS+MB-mediated treatments for neurological conditions, to maximize their benefit-risk ratio.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Box 1

Focused Ultrasound (FUS) and microbubbles (MB).

Ultrasound is widely used in diagnostic imaging as it is non-ionizing and facilitates real-time imaging of anatomical structures within the body. A linear or curvilinear array of transducers is used to transmit and receive short pulses at frequencies between 2–18 MHz. The received signals provide information about the nature and location of internal structures. While some features within the body can be easily distinguished by ultrasound, this is not the case for blood vessels and consequently gas microbubbles (MBs) have been used for over 2 decades as a contrast agent to improve imaging of the vasculature.

Being filled with gas, MB are highly compressible and hence respond strongly to the mechanical perturbations imposed by a sound field. The fluctuating pressure causes the MB to volumetrically oscillate and re-radiate the incident energy at multiple frequencies. This nonlinear response can be detected by an ultrasound transducer and is fundamental to both microbubble imaging and real-time control of BBB opening. In therapeutic applications, the oscillations of the MB are thought to mechanically stimulate BBB opening and thus locally enhance drug uptake.

The attenuation of ultrasound in most tissues increases with frequency via a power law relationship and leads to increased heat deposition due to viscous absorption. Thus, for FUS+MB, lower frequencies (~1 MHz) than those used in imaging are used to prevent off target heating of surrounding tissue especially of bony structures such as the skull. Therapeutic applications also typically employ longer pulses than those used in imaging to increase the probability of generating the desired biological effect.

Outstanding Questions

- Can some of the hallmarks of neurological disease be attributed to neurovascular dysfunction as much as to neuronal dysregulation?
- What are the biological effects of mechanical modulation of the BBB produced by focused ultrasound (FUS) and microbubbles (MB)?
- Clinically, FUS+MB have so far been applied primarily as a treatment for terminal conditions such as glioblastoma. If, however, they are applied in the future to non-terminal CNS diseases, what are the potential long-term adverse effects that should be considered by clinicians and researchers?
- How would a course of several FUS+MB treatments affect the long-term integrity of the BBB?
- Should there be a strategic algorithm or pipeline in place for determining appropriate use of FUS+MB?
- How can the potential adverse effects of neuroinflammation arising from FUS+MB disruption of the BBB be minimized? Can pre- or post-operative strategies be developed to contain or mitigate such effects?

Highlights

- The blood-brain barrier plays both a physical and a physiological “gate keeping” role in maintaining brain homeostasis.
- In recent years, there has been increasing interest in understanding the role of the blood-brain barrier in neurological disorders that were traditionally considered to be neuron-centric, for instance Parkinson’s and Alzheimer’s disease.
- Alternative drug delivery techniques, such as focused ultrasound (FUS), are emerging as powerful tools to bypass the blood-brain barrier and facilitate treatment of neurological conditions.
- To enable widespread clinical use of these techniques, there is an urgent need to investigate and address the associated safety concerns, for example, the consequences of sterile inflammation that may be induced by barrier disruption.

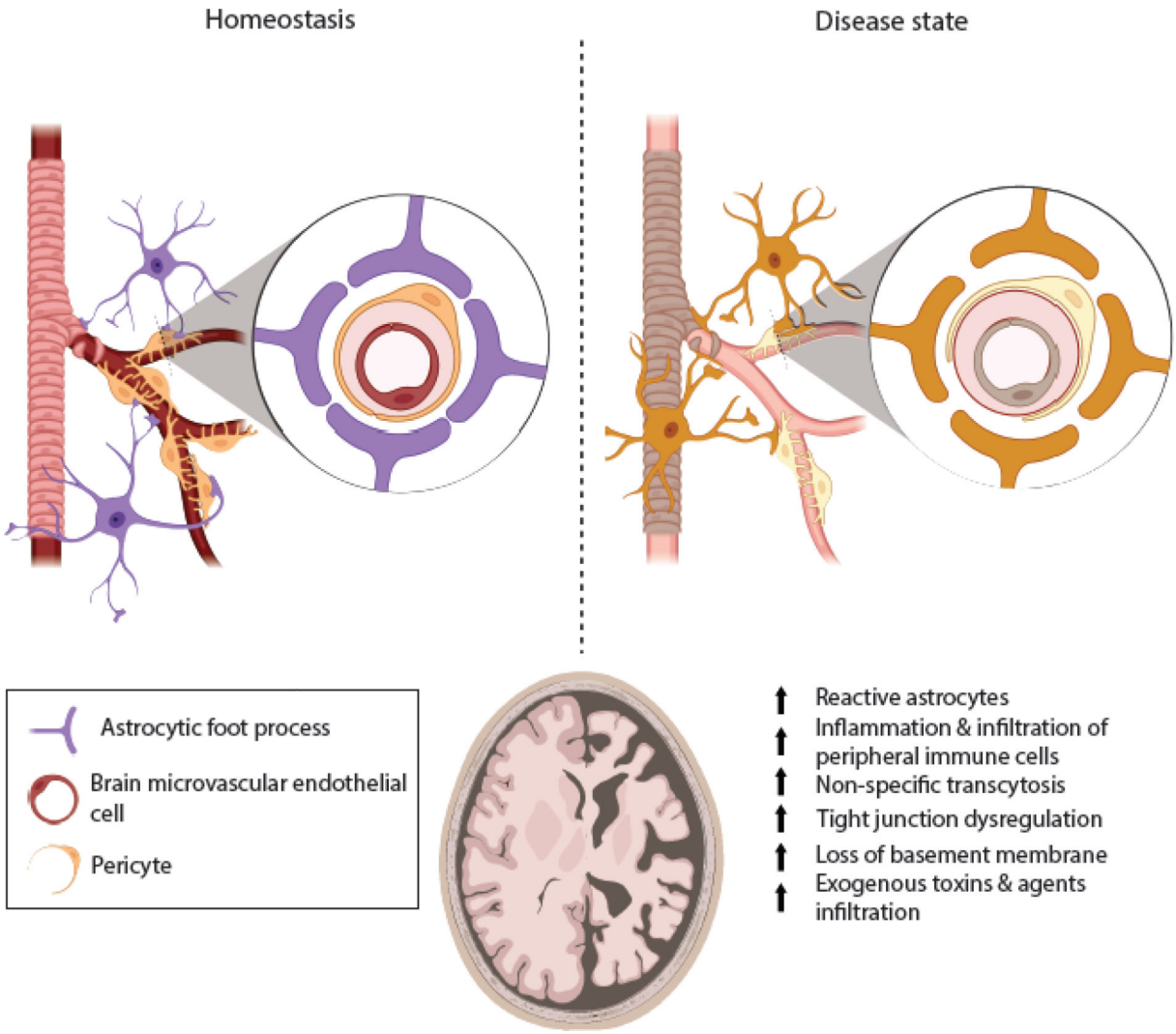

Figure 1. An overview of the cellular composition of the brain vasculature.

The blood brain barrier (BBB) is composed of brain endothelial cells supported by pericytes and endfoot processes of astrocytes. The microvascular endothelial cells form continuous tight junctions with one another, and the astrocytes and pericytes support the vascular network along with the basement membrane lining along the basolateral aspect of the endothelia. In homeostasis, the BBB prevents harmful toxins and agents from entering the central nervous system. This is essential because neurons are especially sensitive to microenvironmental changes. In many neurological diseases, the ability of endothelial cells to form tight junctions is compromised, pericytes' ability to effectively support the vascular network is impaired, and reactive astrocytes signal and interact with microglia, the resident brain "macrophages." There is an increase in local inflammation that leads to further leakage and dysregulation of tight junction complexes, that can allow for chemotaxis of peripheral immune cells. In extreme circumstances, the increased permeability can be so severe that it allows exogenous agents to enter the brain parenchyma, which can be devastating for neurons and the relevant network near the disrupted BBB. Image created through Biorender.

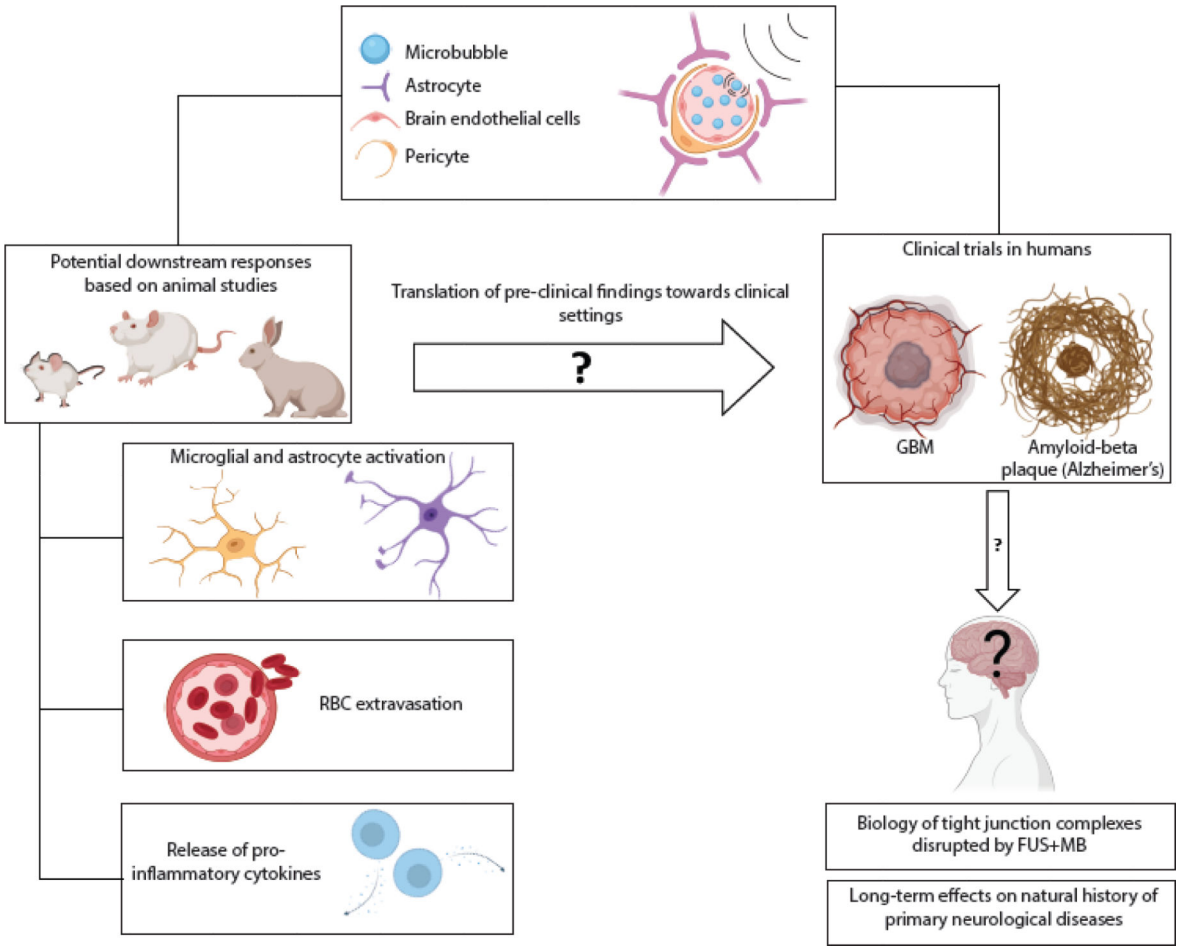

Figure 2. Biological effects of FUS+MB via disruption of the BBB.

The combination of FUS+MB has shown considerable promise as a drug delivery strategy but further understanding of the downstream effects is required. Depending on the ultrasound exposure conditions, a number of biological effects have been observed in preclinical rodent models, including: activation of microglia and astrocytes increasing with increasing acoustic pressure [60]; extravasation of RBCs, which can be minimized through appropriate adjustment of acoustic parameters and/or MB size [51, 56]; and release of cytokines in brain regions contralateral to the hemisphere treated with FUS+MB [46]. It has yet to be seen if these effects occur in humans. To date, clinical studies of FUS+MB applications have focused on treatment of GBM [iv], [vi], dissolution of protein aggregates in Alzheimer's disease [ii] [7], and alleviation of symptoms in Parkinson's disease [iii], [xi] [8]. Further work is needed to examine potential longer-term biological effects, particularly as the range of clinical application is broadened, and clinical trials involve repeated treatments or younger populations [ix], [xiii], [xiv],[xv]. Abbreviations: FUS: focused ultrasound; MB: microbubbles; RBC: red blood cell; GBM: glioblastoma. Image created through Biorender.

[iv] https://clinicaltrials.gov/ct2/show/NCT02253212
[vi] https://clinicaltrials.gov/ct2/show/NCT02343991
[xi] https://clinicaltrials.gov/ct2/show/NCT04370665

[ix] https://clinicaltrials.gov/ct2/show/NCT03782194
[xiii] https://clinicaltrials.gov/ct2/show/NCT04620460
[xiv] https://clinicaltrials.gov/ct2/show/NCT04804709
[xv] https://clinicaltrials.gov/ct2/show/NCT05089786

Table 1.

Overview of clinical trials using FUS+MB for the treatment or diagnosis of CNS disease.

Clinical trial (NIH reference)	Clinical trial phase	Condition of interest	Study description & role of inflammation as modulator or side effect
NCT02343991 [vi]	Phase not applicable	Brain tumors	Evaluate whether FUS can increase passage of tumor-specific biomarkers into the vasculature, improving the quality of liquid biopsy [106]
NCT02253212 [iv]	Phase III	Glioblastoma (recurrent)	Evaluate BBB opening tolerated by patients before delivery of chemotherapeutics; discusses anticancer immune response in the context of other organ-specific cancers (e.g., breast cancer) and studies in other species (e.g., mouse models) in [107]
NCT02986932 [ii]	Phase I	Alzheimer's disease	Reduction of pathological protein aggregate in AD; no mention of inflammation as modulator or in post-treatment evaluation [7]
NCT03321487 [i]	Phase not applicable	Amyotrophic lateral sclerosis	Evaluation of BBB opening in primary motor cortex; MRI imaging show transient disruption via gadolinium perfusion [6]; the authors reported no significant inflammation 30 days post-procedure.
NCT03551249 [vii]	Phase not applicable	Glioma	Establishing safety profile for patients using FUS+MB as first line of therapy (standard chemotherapy); no mention of inflammation as immunomodulator for glioma treatment.
NCT03608553 [iii]	Phase I	Parkinson's disease dementia	Performed BBB opening in parieto-occipito-temporal regions of the patients' brains; no adverse effects reported [8]; no mention of inflammation
NCT03616860 [viii]	Phase I	Glioma	Evaluating FUS+MB to increase quality of liquid biopsy via increasing tumor biomarker perfusion into vasculature through transient BBB opening [108]; no mention of inflammation as possible modulator
NCT03671889 [v]	Phase II	Alzheimer's disease	Evaluation of focal, transient BBB opening in the hippocampus; found indications of perivenous blood-meningeal permeability post-barrier disruption which may be indicative of tissue healing process (in the context of inflammation) [100]
NCT03782194 [ix]	Phase not applicable	Anxiety, obsessive compulsive disorder, posttraumatic stress disorder	Investigate whether usage of FUS pulsation can influence amygdala function to improve emotion regulation
NCT04118764 [x]	Phase not applicable	Alzheimer's disease	Prospective study done with non-human primates in which eosinophil count increased; low acoustic pressure leads to minimal inflammatory cell density [109]
NCT04370665 [xi]	Phase not applicable	Parkinson's disease	Delivering imiglucerase using Exablate MRgFUS system and Definity to open the BBB; no mention of inflammation
NCT04526262 [xii]	Phase not applicable	Alzheimer's disease	Evaluated plaque removal and cognitive functions post-FUS+MB treatment (repeated opening) [110]; no mention of inflammation specific to the study
NCT04620460 [xiii]	Phase not applicable	Schizophrenia	Investigate whether FUS pulsation can modulate cortical function; no mention of immunomodulation as mechanistic target
NCT04804709 [xiv]	Phase I	Progressive diffuse midline glioma (DMG)	Evaluate whether FUS+MB delivery of Panobinostat through transient BBB opening is safe (phase I); no discussion on immunomodulation as possible mechanism
NCT05089786 [xv]	Phase II	Treatment-resistant neurologic and	To evaluate whether FUS can improve clinical measurements in neurological and psychiatric disorders; no discussion of inflammation

[vii] https://clinicaltrials.gov/ct2/show/NCT03551249
[viii] https://clinicaltrials.gov/ct2/show/NCT03616860
[v] https://clinicaltrials.gov/ct2/show/NCT03671889
[x] https://clinicaltrials.gov/ct2/show/NCT04118764
[xii] https://clinicaltrials.gov/ct2/show/NCT04526262

Clinical trial (NIH reference)	Clinical trial phase	Condition of interest	Study description & role of inflammation as modulator or side effect
		psychiatric indications	