Focused Ultrasound-Mediated Blood–Brain Barrier Opening for Neurological Disorders

Subjects: Neurosciences

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Several therapeutic agents for neurological disorders are usually not delivered to the brain owing to the presence of the blood–brain barrier (BBB), a special structure present in the central nervous system (CNS). Focused ultrasound (FUS) combined with microbubbles can reversibly and temporarily open the BBB, enabling the application of various therapeutic agents in patients with neurological disorders.

focused ultrasound | blood–brain barrier | neurological disorders | drug delivery

1. Alzheimer’s Disease

The incidence of Alzheimer’s disease (AD), the most representative neurodegenerative brain disease, is steadily increasing as the aging population increases. However, only drugs that can alleviate and delay symptoms are currently being used, and no specific treatment methods or therapeutic agents [1] have been developed yet. Over the past decades, several clinical trials have been conducted with various targets, focusing on these two clinical indications: amyloid beta plaques and neurofibrillary tau tangles [2]. However, all clinical trials have failed; only Aducanumab, which targets amyloid-β (Aβ) plaque removal, has shown a therapeutic effect, but it is controversial due to side effects [3][4]. Although the amyloid hypothesis remains controversial, since the accumulation of Aβ is a representative pathological hallmark of AD, numerous therapeutic studies targeting Aβ have been conducted.

The first preclinical study on FUS for AD aimed to deliver anti-Aβ antibodies targeting amyloid plaques into the brain by a BBB opening. Consequently, anti-Aβ antibodies bound to the Aβ plaques and rapidly reduced the plaque pathology [5]. Subsequently, research on delivering therapeutic agents through FUS-mediated BBB opening in patients with AD has gained attention [6][7][8][9][10][11][12]. Interestingly, studies have reported that amyloid pathology [6][13][14][15][16] and phosphorylated tau [17][18] are reduced only by FUS-induced BBB opening without specific drug delivery. Treatment delivery via FUS-mediated BBB opening also affected memory recovery in AD animal models [14][19][20][21][22]. Research studies on various biological changes by FUS-mediated BBB opening are ongoing. However, for FUS to be a promising non-pharmacological treatment delivery method for AD, further research is needed on why amyloid is reduced and cognitive function is restored. FUS induces the activation of microglia and astrocytes, which may increase phagocytosis of the amyloid plaques [13][14][23]. Recently, a study confirming the therapeutic effect in an AD mouse model (5×FAD) by combining FUS and Aducanumab was reported [24]. Aducanumab, a monoclonal antibody targeting fibril forms and beta-amyloid oligomer, has been proven effective since receiving FDA approval in 2021. However, due to side effects, debate continues as to whether or not it should be used.

In conclusion, combined treatment with FUS and Aducanumab reduced amyloid plaque levels, increased hippocampal neurogenesis, and restored cognitive function. Here, FUS activated phagocytic microglia and increased the number of astrocytes associated with amyloid plaques. This suggests that FUS can induce a reduction in amyloid plaques through phagocytosis. In addition, an RNA sequencing analysis showed that the combined treatment with FUS and Aducanumab upregulated neuroinflammation signaling, phagosome formation, reelin signaling, and CREB signaling [24]. The immunomodulatory effect of FUS, such as the activation of various innate immune cells, plays a vital role in reducing amyloid plaques [19]. Regarding the recovery of cognitive function by FUS, the increase in hippocampal neurogenesis [25][26][27][28] or synaptic plasticity [27][29] may play a role here, but further research is needed on this topic. The researchers summarized the most relevant preclinical studies on FUS-mediated BBB opening in AD (**Table 1**).

Table 1. Recent preclinical studies on focused ultrasound-mediated blood–brain barrier opening in Alzheimer's disease.

Authors, Year of Publication	Animal Model	FUS Parameters	Target Region	Main Results
Xhima (2020) [7]	TgCRND8 mice	CF:1.68 MHz PRF:1 Hz TD:120 s AP: Maintained after decreasing to 25% based on subharmonic emissions	Basal forebrain	Delivery of D3 (peptidomimetic agonist of TrkA) to the basal forebrain via FUS activated the TrkA-related signaling cascades and increased cholinergic neurotransmission.
Dubey (2020) [10]	TgCRND8 mice	CF:1.68 MHz PRF:1 Hz TD:120 s AP: 0.23 MPa (feedback controller)	Cortex and hippocampus	IVIg-FUS significantly increased neurogenesis. FUS alone and IVIg alone significantly reduced amyloid plaques. IVIg-FUS affects neurogenesis through the downregulation of TNF-α.
Deng (2021) [30]	APP/PS1 transgenic mice	CF:1 MHz PRF:10 Hz TD:60 s AP:0.6 MPa	Posterior 3.5 Lateral 3.5 Ventral 3.5 (mm)	Proved the possibility of extracting exosomes from astrocytes through ultrasonic stimulation. Astrocyte-derived exosome was delivered to the brain after opening the BBB to confirm the amyloid clearance effect.
Feng (2021) [31]	Sprague-Dawley rats Aβ (1–40) injection model	CF:1 MHz TD:60 s AP:0.8 MPa	Hippocampus	As a result of the delivery of MpLXSN-BDNF (modified MB with retrovirus-BDNF) through FUS, cognitive function is improved, and BDNF restores synaptic loss.

Authors, Year of Publication	Animal Model	FUS Parameters	Target Region	Main Results
Leinenga (2021) [32]	APP23 transgenic mice	CF:1 MHz PRF:10 Hz TD:6 s AP:0.7 MPa	Whole brain	The combined treatment of scanning ultrasound and Aducanumab induced the effect of reducing amyloid plaques in the hippocampus and restored cognitive function.
Poon (2021) [33]	TgCRND8 mice	CF:1 MHz PRF:1 Hz TD:120 s AP:0.28–0.55 MPa	Hippocampi and cortices	FUS-mediated BBB opening treatment three to five times biweekly did not induce neutrophil recruitment or phagocytosis of amyloid plaques.
Sun (2021) [34]	Aged APP/PS1dE9 mice	CF:278 kHz PRF:2 Hz TD:100 s AP:0.33 MPa	Hippocampi	FUS increased the delivery rate of 07/2a mAb (Fc-competent anti-pGlu3 Aβ monoclonal antibody) to the brain by 5.5 times. Co-treatment with FUS and 07/2a mAb induces greater effects on learning and memory recovery and increases synaptic puncta.
Luo (2022) [35]	Kunming mice Aβ1–42 injection model	CF:1 MHz PRF:1 Hz TD:120 s Voltage: 200 mV	Hippocampus	FUS-Gastrodin treatment restored memory and alleviated neuropathology. FUS-Gastrodin reduced Aβ, tau, and P-tau and upregulated BDNF, synaptophysin, and PSD-95 in the hippocampus.
Bathini (2022) [36]	APP/PS1dE9 transgenic mice	CF:278 kHz PRF:2 Hz TD:100 s AP:0.33 MPa	Cortex and hippocampus	07/2a mAb (anti-pyroglutamate-3 Aβ antibody) delivered with FUS resulted in a 5- to 6-fold increase in the brain-to-blood antibody ratio after 4 and 72 h. FUS-07/2a mAb enhanced the immunoreactivity of resident Iba1+ and phagocytic CD68+ microglia.
Bajracharya (2022) [37]	K3 mice (human 1N4R tau)	CF:1 MHz PRF:10 Hz TD:6 s AP:0.5 MPa	Whole brain	Repeated FUS-BBB opening reduces tau inclusions. FUS-BBB opening mediates delivery of RNF5 (tau-specific monoclonal antibody) increase brain uptake and accumulates in unclear cells within the pyramidal layer.
Kong (2022) [24]	5×FAD mice	CF:0.5 MHz PRF:1 Hz TD:120 s AP:0.25 MPa	Hippocampi	Combined therapy of FUS and Aducanumab decreases amyloid deposits, increases neurogenesis,

o the loss of dopaminergic neurons. PD is neuropathologically characterized by proteinaceous inclusions called Lewy bodies [38]. Notably, as many studies have reported that α-synuclein plays a direct role in disease development, PD is classified as α-synucleinopathies [39]. Currently, there are no clear treatments to slow or alleviate the progression of

Authors, Year of Publication	Animal Model	FUS Parameters	Target Region	Main Results
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has been confirmed [43][44][45]; however, one study was discontinued due to safety concerns in clinical trials [46]. Animal studies of GDNF gene delivery by FUS began in PD and have highlighted the possibility of effective gene therapy [47][48][49].

AP, acoustic pressure; BDNF, brain-derived neurotrophic factor; CF, center frequency; FUS, focused ultrasound; MB, microbubble; PRF, pulse repetition frequency; PSD-95, postsynaptic density protein-95; TD, train duration; TH, tyrosine hydroxylase.

Since neurturin has been found to have neuroprotective and neuro-regenerative effects on dopaminergic neurons [50], the FUS-based delivery of neurturin has been studied to find an alternative to GDNF [51][52]. Recently, recombinant adeno-associated viral (rAAV) vectors have received much attention as a tool for gene delivery to the brain. The technology of delivering rAAV using FUS-mediated BBB permeability and expressing the delivered gene has already been examined [53]. Accordingly, recent studies on PD models using FUS mainly involve gene delivery using rAAV. While there are many studies on delaying disease symptoms by delivering various therapeutic agents using FUS, there is a lack of preclinical research studies on α-synuclein-based PD models. The most relevant preclinical studies on FUS-mediated BBB opening in PD were summarized (**Table 2**).

Table 2. Recent preclinical studies on focused ultrasound-mediated blood–brain barrier opening in Parkinson's disease.

Authors, Year of Publication	Animal Model	FUS Parameters	Target Region	Main Results
Ji (2019) [54]	C57BL/6 mice MPTP	CF:1.5 MHz PRF:10 Hz TD:60 s AP:0.45 MPa	Striatum and substantia nigra	FUS-Intranasal delivery increased TH immunoreactivity and improved motor control function.
Lin (2020) [55]	Balb/c mice MPTP	CF:1 MHz PRF:10 Hz TD:180 s Voltage:85 V	Substantia nigra	BDNF or GDNF gene delivery through the UTMD system induces a neuroprotective effect. However, combined with the GDNF/BDNF gene delivery it did not produce benefits compared with individually delivering BDNF or GDNF genes.
Yan (2021) [56]	C57BL6 mice MPTP	CF:1 MHz PRF:1 Hz TD:60 s AP:0.24–0.45 MPa	Cortex, striatum, and substantia nigra	Improves therapeutic efficacy by increasing the delivery rate of encapsulated curcumin through FUS.
Yuhong (2022) [57]	C57BL/6J mice MPTP	CF:1 MHz PRF:1 Hz TD:60 s Voltage:100, 150, 200 mV	Striatum	FUS increased the delivery rate of gastrodin, which induces neuroprotective effects, by 1.8-fold. FUS-Gastrodin treatment increased the expression levels of Bcl-2, BDNF, PSD-95,

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				and synaptophysin protein and decreased the levels of caspase-3 in the striatum.
Trinh (2022) [58]	Sprague-Dawley rats	CF:1 MHz PRF:1 Hz TD:120 s AP:0.4 MPa	Striatum and substantia nigra	FUS-induced BBB permeability in the striatum and substantia nigra. SIRT3-myc (viral vector gene therapies for PD) was expressed only in the striatum.

AP, acoustic pressure; Bcl-2, B-cell lymphoma-2; BDNF, brain-derived neurotrophic factor; CF, center frequency; FUS, focused ultrasound; GDNF, glia cell line-derived neurotrophic factor; MPTP, neurotoxin 1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine; PD, Parkinson's disease; PRF, pulse repetition frequency; PSD-95, postsynaptic density protein-95; TD, train duration; TH, tyrosine hydroxylase; UTMD, ultrasound-targeted microbubble destruction.

Glioblastoma is the most aggressive brain tumor with a high recurrence rate and poor prognosis despite treatments such as resection, radiotherapy, and chemotherapy [59]. The blood–tumor barrier (BTB) is created by the often heterogeneous disruption of the BBB within the tumor due to aberrant angiogenic signaling. As the delivery of anticancer drugs is limited despite the irregular leakiness of the BTB, quantitative drug delivery through FUS-mediated BBB opening is required [60]. Many previous studies on drug delivery by FUS have involved patients with brain tumors. Doxorubicin is a chemotherapeutic agent that inhibits cell growth and induces apoptosis in malignant glioma cells; however, it is not commonly used because it cannot cross the BBB. In 2007, Treat et al. delivered doxorubicin to a tumor in the brain via FUS-mediated BBB opening, indicating that this drug could be a viable treatment option [61]. Until now, various therapeutic agents have been used to treat glioblastomas, and FUS-mediated BBB opening technology is being developed. In the early days of FUS research, unencapsulated drugs such as the common anticancer drug temozolomide (TMZ) [62][63], carmustine (BCNU) [64], and immunostimulatory interleukin-12 (IL-12) [65][66] were mainly used.

Brain metastasis represents an important predictor of mortality for various non-brain cancers such as breast cancer. Like primary brain tumors, brain metastases do not have an intact BBB, but most therapeutics still have lower intra-tumoral bioavailability than non-brain tumors [67]. FUS studies have continued to treat metastatic brain tumors as well as primary brain tumors. In 2012, there was a study confirming the therapeutic effect by delivering Trastuzumab based on FUS-BBB opening in a breast cancer brain metastases model [68]. Additional research reported in 2016 demonstrated that the administration of trastuzumab and pertuzumab in a brain metastasis mouse model of breast cancer inhibited the growth of brain metastasis when used with FUS, compared to chemotherapy alone [69].

Whether it is a primary brain tumor or a metastatic brain tumor, the critical factor in the tumor microenvironment is to what extent the anticancer drugs could be delivered into the target region. It has been reported that the delivery of chemotherapeutic agents with small molecular weights to the brain tumor microenvironment is approximately 3.9-fold higher under FUS-mediated BBB opening conditions [70]. This enhanced delivery rate has been shown to increase median survival by approximately 30% compared to chemotherapy alone.

However, efflux transporters such as Pgp are overexpressed in cancer cells and prevent the uptake of anticancer drugs into the cells, resulting in resistance to them. FUS-mediated BBB opening temporarily inhibits Pgp expression, thereby preventing drug efflux and interfering with functional components of the BBB [71]. Additional research is needed on efflux transporter inhibitors targeting cancer cells. In addition to unencapsulated drugs, studies have reported that tumors (metastatic breast cancer) can be effectively controlled by delivering natural killer

cells under BBB opening [72]. Furthermore, studies on suppressing brain tumors by delivering patient-specific antibodies or complexes loaded on short-hairpin RNA-liposomes have also been previously reported [73]. Since then, several studies have been conducted to enhance the safety and efficiency of tumor treatment by delivering encapsulated therapeutics through the conjugation of existing drugs or genes with improved MB, virus, and nanoparticles [74][75][76][77]. As immunotherapy is a critical issue in neuro-oncology, additional research on immunotherapy using FUS-mediated BBB opening is expected to become more active in the future. The most relevant preclinical studies on FUS-mediated BBB opening in brain tumors were summarized (**Table 3**).

Table 3. Recent preclinical studies on focused ultrasound-mediated blood–brain barrier opening in brain tumors.

Authors, Year of Publication	Animal Model	FUS Parameters	Target Region	Main Results
McDannold (2020) [78]	Sprague-Dawley rats F98 glioma	CF:230 kHz PRF:1.1 Hz TD:55 s AP:119–186 kPa	Striatum (Tumor)	It was confirmed that the ExAblate Neuro low-frequency clinical TcMRgFUS system could stably open the BBB in a rat model. Although delivery of irinotecan to the brain was not neurotoxic, it was not effective in prolonging survival or reducing the growth of gliomas.
Curley (2020) [75]	athymic nude mice U87 GBM	CF:1.1 MHz DC:0.5% TD:120 s AP:0.45–0.55 MPa	Striatum (Tumor)	Interstitial fluid transport in brain tumors is increased by FUS. FUS increased the dispersion of directly injected brain-penetrating nanoparticles through tumor tissue by >100%.
Englander (2021) [79]	B6 mice PDGF-B + PTEN−/−p53−/− murine glioma	CF:1.5 MHz PRF:5 Hz TD:120 s AP:0.7 MPa	Pons (Tumor)	FUS increased the delivery rate of etoposide into the tumor site more than five times compared to the control group, but there was no difference in survival rate or inflammation.
Sheybani (2021) [80]	C57BL/6 mice GL261 glioma	CF:1.1 MHz DC:0.5% TD:120 s AP:0.4 MPa	Striatum (Tumor)	[89Zr]-mCD47 (phagocytic immunotherapy) delivery with repeated FUS can significantly constrain tumor outgrowth and extend survival rate.
Ye (2021) [81]	Swiss-Webster mice GL261 glioma	CF:1.5 MHz PRF:5 Hz TD:60 s AP:0.43 MPa	Brain stem (Tumor)	FUS-mediated intranasal delivery increased the delivery rate of anti-PD-L1 antibodies to the brain stem by 4.03-fold.
Chen (2021) [82]	Fisher rats C6 glioma	CF:400 kHz	Caudate putamen	CD4+ (helper TILs) and CD8+ (cytotoxic TILs) immunogenic responses were significantly

Authors, Year of Publication	Animal Model	FUS Parameters	Target Region	Main Results
		PRF:1 Hz TD:120 s AP:0.81 MPa	(Tumor)	increased after 7 days of FUS treatment.
Moon (2022) [83]	BALB/c nude mice U87 GBM	CF:1 MHz PRF:1 Hz TD:60 s AP:1 W/cm^2	Cerebral hemisphere	Sonosensitive liposome-encapsulating doxorubicin enhances permeability by FUS-mediated BBB opening. The GBM cytotoxicity of IMP301-DC was significantly increased.
Sheybani (2022) [84]	C57BL/6 mice GL261 glioma	CF:1.1 MHz PRF:1 Hz TD:120 s AP:0.4–0.6 MPa	Striatum (Tumor)	FUS-mediated BBB opening in gliomas transiently induces inflammatory effects.

AP, acoustic pressure; BBB, blood–brain barrier; CF, center frequency; DC, duty cycle; FUS, focused ultrasound; GBM, glioblastoma; PRF, pulse repetition frequency; TD, train duration; TILs, tumor-infiltrating lymphocytes.

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