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Emerging Applications of Therapeutic Ultrasound in Neuro-Oncology: Moving Beyond Tumor Ablation

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Abstract

Transcranial focused ultrasound (FUS) can noninvasively transmit acoustic energy with a high degree of accuracy and safety to targets and regions within the brain. Technological advances, including phased array transducers and real-time temperature monitoring with magnetic resonance (MR) thermometry, have created new opportunities for FUS research and clinical translation. Neuro-oncology, in particular, has become a major area of interest, as FUS offers a multifaceted approach to the treatment of brain tumors. FUS has the potential to (1) generate cytotoxicity within tumor tissue, both directly via thermal ablation and indirectly through radiosensitization and sonodynamic therapy; (2) enhance the delivery of therapeutic agents to brain tumors by transiently opening the blood-brain barrier and/or improving distribution through the brain extracellular space; and (3) modulate the tumor microenvironment in order to generate an immune response. In this review, we describe each of these applications for FUS, the proposed mechanisms of action, and the preclinical and clinical studies that have set the foundation for utilizing FUS in neuro-oncology.

Keywords

Blood-brain barrier; brain neoplasms; drug delivery; extracellular space; high-intensity focused ultrasound; immunomodulation

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INTRODUCTION

Transcranial focused ultrasound (FUS) has been investigated for over 60 years, with seminal studies by John Lynn and Tracy Putnam in the 1940s and William and Francis Fry in the 1950s establishing the potential of applying ultrasound to cerebral tissue.^{1–3} Early explorations of FUS for clinical use, however, were limited by the need for a craniectomy due to beam distortion and energy absorption by the intact skull. In the 1990s, hemispheric phased arrays of transducers were developed along with software that corrects for the phase aberrations produced by the variable thickness of the skull. This revolutionized the field by allowing for the noninvasive transmission of ultrasound beams to focal regions across irregular bone and tissue interfaces.⁴ Additionally, advances in magnetic resonance (MR) imaging, specifically the development of MR thermometry, allowed temperature changes to be visualized in real time, thereby enabling safety monitoring and confirmation of the energy being applied at the acoustic focus.⁵

Once these technologies were incorporated, FUS research in the neurosciences increased dramatically, building on prior successes in treating non-neurological disorders such as uterine fibroids. Ultimately, the existing clinical MR-guided focused ultrasound (MRgFUS) devices were modified for the non-invasive application of focused exposures in the human brain. MRgFUS recently completed a clinical trial for the treatment of essential tremor (NCT01304758) and is currently being evaluated for Parkinson's disease (NCT01772693, NCT02246374, NCT02263885), depression (NCT02348411), epilepsy (NCT02151175), neuropathic pain (NCT01699477), and acute brain injury (NCT02522429). The technology has numerous other potential therapeutic applications, including the treatment of stroke, obsessive compulsive disorder, Alzheimer's disease, trigeminal neuralgia, and hydrocephalus (for a review, see Medel et al⁶). Neuro-oncology, in particular, is receiving renewed attention, as MRgFUS provides new options for the targeted, noninvasive treatment of brain tumors, in contrast to more invasive thermal approaches that are emerging as viable treatment options.^{7,8} This article reviews the preclinical and clinical work exploring MRgFUS for (1) generating cytotoxicity within tumor tissue, (2) enhancing the delivery or activity of therapeutic agents, and (3) modulating the tumor microenvironment to enhance immune recognition and clearance (Figure 1 and Table 1).

BIOLOGICAL EFFECTS OF ULTRASOUND—MECHANISMS OF ACTION

Heat

FUS utilizes either spherically curved transducers or phased arrays of smaller single-element transducers to focus energy into a small volume at a particular distance from the transducer. At the focal zone, the spatial intensity is several orders of magnitude higher than in the pre-focal region. As a result, the effects of the ultrasound beam are generally limited to the focal point, where the high rate of energy deposition results in the efficient generation of heat and subsequent temperature elevations that can reach up to 60°C in seconds (for a comprehensive review, see Haar and Coussios⁹). For the generation of thermal lesions, these can be very sharply delineated from a homogenous thermal dose, with lethal and sublethal effects being separated by only several cell thicknesses.^{10,11} The diameter of the focal zone is typically around 1 mm, and the length can range from 5 to 20 times this dimension. These

parameters are dependent on the specific dimensions of the transducer (ie, the diameter and the radius of curvature) as well as the frequency.¹² Due to the small volume of the focal zone relative to the regions that are typically treated (eg, a mid-sized uterine fibroid, which is 50–100 mm³), a large number of contiguous or overlapping treatments are required, leading to potentially long treatment times.^{11,13}

Alternatively, pulsed exposures with short duty cycles (eg, 5%–10%) reduce the overall temporal average intensity, typically generating minimal temperature elevations on the order of 4°C–5°C.^{14,15} Pulsed focused ultrasound (pFUS) therefore allows for the non-thermal, mechanical effects of ultrasound to predominate. These include acoustic cavitation, acoustic radiation forces, and acoustic streaming.

Acoustic Cavitation

Ultrasound is a pressure wave consisting of positive (compressive) and negative (rarefactive) components, where the negative component may enable the expansion of small, stabilized gas-filled “cavities” or bubbles within a liquid medium. As the ultrasound exposure continues, the bubbles oscillate, with their diameter varying with the alternating pressure field. The bubbles may continue to oscillate in a stable fashion, (ie, non-inertial cavitation), but if the pressure wave amplitude increases, the bubbles collapse (ie, inertial cavitation), producing violent shock waves and high-velocity jets, with substantial mechanical and potentially detrimental effects on the surrounding tissues.¹⁶

Radiation Forces and Acoustic Streaming

When an ultrasound beam transfers momentum to a reflecting or absorbing surface, a small, steady, unidirectional force is produced along the direction of the beam. These radiation forces, if large enough, produce displacements in the tissue at the focal point, though not in the surrounding tissues. The resulting non-uniformity in displacement may produce strain in the tissue.¹⁷ In a similar fashion, acoustic streaming results from the radiation forces that take place specifically within a liquid medium. The resulting circulation set up by the acoustic field may enhance convection as well as produce shear forces that can cause tissue damage.^{18,19}

CYTOTOXICITY

Through the mechanisms described above, FUS may produce a variety of effects that directly or indirectly destroy tumor tissue. Early on, investigators realized that the temperature elevations at the focal zone can ablate tumor cells, particularly deep-seated tumors that are difficult to access surgically. These include tumors in the liver,²⁰ prostate,²¹ and brain,²² as well as bone metastases.²³ More recently, however, ultrasound has been proposed as an adjuvant therapy that contributes to cytotoxicity via subtler processes, including radiosensitization²⁴ and sonodynamic therapy (SDT).^{25–27}

Thermal Ablation

Heat deposition resulting in thermal ablation is the most direct mechanism by which FUS can be used to treat brain tumors. Continuous exposures result in high rates of energy

deposition, producing progressive elevations in temperature of tens of degrees Celsius. At temperatures above 55°C, cellular death occurs as a result of coagulative necrosis, accompanied by protein denaturation and the disruption of cellular membranes.^{28,29}

FUS is now commonly used for the ablation of uterine fibroids³⁰ and prostate cancer.³¹ In a meta-analysis involving 1594 patients in 10 countries, treatment of uterine fibroids with MRgFUS resulted in moderate reductions in fibroid volume (most studies reported reductions of 10%–30%), and fibroid-associated symptom severity improved significantly.³² Similar results exist for the treatment of prostate cancer with FUS, which is carried out worldwide. Mid- and long-term progression-free survival rates following sonication are ~70%, and negative postoperative biopsies occur in 80% of cases.^{33,34}

The treatment of brain tumors with FUS, however, has been limited to small case series (Table 2). In the early 2000s, Ram et al²² used MRgFUS to treat 3 patients with recurrent glioblastoma (GBM). Although post-treatment imaging and histopathology revealed evidence of thermocoagulation in 2 of the 3 tumors, a craniectomy was required prior to sonication in order to create an acoustic window. At the time, this prerequisite negated one of the main attractions of FUS as a tool for ablation—its noninvasive nature. Additionally, one of the patients developed a secondary focus of thermocoagulation along the sonication path, resulting in a mild left hemiparesis. As a result, the device was later modified to provide temperature measurements along the path of the ultrasound beam, to monitor for heating of the intervening, peritumoral tissue.

More recently, McDannold et al³⁵ reported their experience treating 3 patients with inoperable GBM as part of a phase I clinical trial. Although this was the first group to successfully focus an ultrasound beam through an intact cranium, the version of the device being used at the time did not provide enough power to reach the temperature threshold for coagulative necrosis. Furthermore, a fourth patient experienced an intracranial hemorrhage for unclear reasons, resulting in closure of the study.⁶

Currently, 3 phase I clinical trials (NCT01698437, NCT00147056, NCT01473485) are ongoing to verify the safety of the device and the feasibility of thermal ablation of brain tumors. In order to minimize the risk of intracranial hemorrhage, patients with a bleeding diathesis are currently excluded from the trials, as are those with vascular tumors. Furthermore, in order to maintain precise control over the energy deposition along the ultrasound beam path, exclusion criteria also include the presence of cystic areas adjacent to the tumor, clips or implants in the sonication path, and dural patches or skull reconstructions. Signs of intracranial hypertension or tumoral mass effect are contraindications to MRgFUS as well, given the concern that sonication may result in a transient increase in cerebral edema. These Phase I trials are a critical step toward the translation of MRgFUS into clinical practice.

Radiosensitization

Although FUS can achieve cytotoxicity directly via thermal ablation, it can also do so indirectly by sensitizing brain tumors to radiotherapy via a hyperthermia-based mechanism. Radiation is an integral part of the current standard of care for patients with GBM and other

brain tumors, which commonly involves maximum safe surgical resection followed by adjuvant radiation and chemotherapy.³⁶ Additionally, whole brain radiation therapy followed by a stereotactic radiosurgery (SRS) boost plays an important role in the management of patients with brain metastases.^{37,38}

Non-destructive hyperthermia (to approximately 42°C) has become well established as a means of enhancing the effects of radiation therapy, with numerous randomized trials demonstrating its efficacy in a variety of tumor types.^{39–42} Hyperthermia is thought to sensitize cancer cells to the effects of radiation by preventing the repair of DNA damage and by inducing tumor reoxygenation.^{43,44} Hyperthermia preferentially affects cells in the S phase of the cell cycle, when they are most radioresistant.⁴⁵ Recent evidence also suggests that hyperthermia may have a specific effect on glioma stem-like cells through the inhibition of AKT signaling.⁴⁶

A randomized phase II/III study involving 79 patients with focal GBM explored the radiosensitizing effects of hyperthermia.⁴² Following surgical resection, patients underwent fractionated external beam radiation to a dose of 59.4 Gy with concomitant oral hydroxyurea. Patients with an “implantable” tumor at the completion of treatment were then randomized to interstitial brachytherapy with or without microwave-based hyperthermia. Hyperthermia was associated with significantly longer time to progression ($P = .045$) and survival from date of diagnosis ($P = .02$). Median survival was 76 weeks vs 85 weeks, and 2-year survival was 15% vs 31% for the control vs hyperthermia groups, respectively.

Historically, however, the challenge has been to efficiently and safely produce low-level hyperthermia within brain tissue. One of the more recent technologies to emerge has been MR-guided laser interstitial thermal therapy, which utilizes a solid-state diode laser to generate a focal region of hyperthermia. Despite accumulating evidence regarding its efficacy, the technology still requires the laser probe to be stereotactically implanted into the tumor (for a review, see Hawasli et al⁴⁷).

FUS, however, represents a noninvasive alternative; utilizing the pulsed sequences described above, low levels of hyperthermia can be achieved in focal, deep brain targets. As early as 1991, FUS-induced hyperthermia was studied as an adjunct to radiation therapy for the treatment of brain tumors.²⁴ This phase I study involved 15 patients with grade III astrocytoma ($n = 2$) or GBM ($n = 13$). Five of the patients presented with primary tumors and underwent surgical debulking, while 10 patients presented with tumor recurrence following prior treatment. Following a craniectomy and the placement of thermocouple probes to measure the temperature in the region of interest, patients underwent weekly sonication sessions while receiving daily external beam radiotherapy. Hyperthermia was achieved using a 1.7 MHz curved transducer and a scanning mechanism. Over the course of 51 treatments, 41.9% of the thermocouple probes reached the target temperature of 42°C. The highly heterogeneous structure of the tumors was felt to result in non-uniform power deposition, thereby presenting a challenge to precise control over the procedure. Other limitations included impedance mismatches produced by aerated gelatin sponges and dural substitutes, attenuation by the temporalis muscle during the treatment of temporal lobe tumors, and the mitigating effect of bloodflow on heating in highly perfused tumors.

Additionally, the need for a craniectomy as well as invasive temperature probes added surgical risk and morbidity to the procedure. However, with the recent advances in MRgFUS and MR thermometry, hyperthermia can now be achieved non-invasively and has become an even more attractive option for accomplishing radiosensitization.

Sonodynamic Therapy

An alternative approach to achieving cytotoxicity relies on sensitizing agents that absorb energy and produce reactive sonochemical species, ultimately resulting in cellular damage. Initially using light of particular wavelengths to activate these agents, photodynamic therapy (PDT) has been explored as a therapeutic option for brain tumors. However, light has poor penetration through most tissues (typically, a few millimeters), thereby requiring PDT to be applied intraoperatively or with interstitial fiberoptic sources.^{48,49} As a result, ultrasound is now being explored as a means of activating sensitizing agents and generating cytotoxicity—a process called sonodynamic therapy. FUS represents an attractive alternative to light-based activation, as it is not restricted by the same tissue penetration limitations.

The cytotoxic effects of combining ultrasound with sonosensitizers have been studied since 1989.⁵⁰ In particular, pFUS has a minimal effect on temperature, and at low power is unlikely to damage cells on its own. Combined with a sonosensitizer, though, pFUS can produce significant cytotoxicity via a cavitation-based mechanism. The energy released by acoustic cavitation bubble collapse activates sonosensitizers and produces free radicals and singlet oxygen. These, in turn, trigger a chain reaction that culminates in lipid peroxidation and cytotoxicity on a cellular scale (for a review, see Chen et al⁵¹ and Rosenthal et al⁵²).

A number of sonosensitizers have been investigated. Porphyrin derivatives represent one of the most widely studied groups of sensitizing agents; hematoporphyrin monomethyl ether (HMME), protoporphyrin IX (PpIX), and ATX-70 have been shown to have a sonodynamic effect in a variety of cancer types, including sarcoma,⁵⁰ hepatocellular carcinoma,⁵³ osteosarcoma,⁵⁴ and endometrial cancer.⁵⁵ Additionally, xanthenes, various chemotherapeutic agents (in particular, doxorubicin), second generation fluoroquinolones, and polyhydroxy fullerenes have all been shown to enhance the cytotoxic effects of ultrasound,⁵¹ although studies in brain tumor models are lacking.

With respect to brain tumors specifically, only a handful of sonosensitizers have been explored. Rose Bengal, for instance, has been shown to have a sonodynamic effect in rats implanted with C6 glioma cells²⁶ but is limited by low levels of specific accumulation in tumor tissue. In particular, 5-aminolevulinic acid (5-Ala) has generated significant interest as a sensitizing agent. 5-Ala is metabolized to PpIX, an endogenous fluorescent bioproduct, as part of the heme biosynthesis pathway. In malignant glioma cells, but not in healthy brain cells, exposure to 5-Ala results in tumor-specific accumulation of PpIX as a result of alterations in enzymes and cell transporters involved in heme biosynthesis. These unique features have proven useful for the intraoperative discrimination of tumor and normal tissue in the operating room,⁵⁶ as well as 5-Ala-based PDT.⁵⁷

More recently, 5-Ala has also been investigated as a potential sonosensitizer. Ohmura et al²⁷ first determined a safety threshold in the normal rat brain; when applying these sonication

parameters to an orthotopic C6 glioma rat model 3 hours after the oral administration of 5-Ala, tumor volumes were smaller than in rats undergoing a sham operation, undergoing sonication alone, or receiving 5-Ala alone (Figure 2). The surrounding brain tissue was left undamaged. These results were later confirmed by Jeong et al²⁵ using a lower ultrasound intensity. These early results are encouraging and warrant further research.

DRUG DELIVERY

While cytotoxicity is a natural goal of any oncologic therapy, MRgFUS is a versatile tool, with applications that extend beyond tumor ablation. A particularly well-studied area of research is the use of MRgFUS for enhancing drug delivery to brain tumors. Therapeutic agents that are systemically delivered must overcome a number of hurdles before reaching their targets within a brain cancer cell. These include the blood-brain barrier (BBB) and the brain tissue/tumor penetration barrier, each of which may be overcome by FUS.

Blood-Brain Barrier Disruption

The first (and most widely studied) obstacle to drug transport across the cerebral vasculature is the BBB, which is formed by specialized endothelial cells that are linked by tight junctions and lack fenestrations. Only small, lipophilic molecules less than 400 Daltons in size are capable of passively diffusing across the BBB. While this serves a protective function, it also prevents the vast majority of therapeutic agents from passively crossing the BBB, resulting in inadequate concentrations within the brain and increased systemic toxicity. Although the BBB is disrupted in many gliomas, it often remains intact at the periphery of the tumor, where invading tumor cells are interspersed with healthy brain cells. Delivering treatments to these invasive cells remains a significant challenge (for a review, see Woodworth et al⁵⁸ and van Tellingen et al⁵⁹).

Current strategies to manipulate or disrupt the BBB include the use of chimeric peptides,⁶⁰ intra-arterial osmotic agents,⁶¹ alkylated alcohols,⁶² pro-inflammatory cytokines,⁶³ and synthetic bradykinin analogs.⁶⁴ However, some of these agents result in widespread and nonspecific BBB breakdown, allowing neural tissue to become exposed to toxic components of the blood. Others, such as chimeric peptides, are more specific but highly inefficient.

A promising alternative approach is to use MRgFUS to mechanically open the BBB in a targeted, localized region. The potential for acoustic energy to open the BBB was demonstrated as early as the 1950s, when trypan blue staining was identified in a sonicated region of the brain, without evidence of discrete damage.⁶⁵ With the recent advances in MRgFUS technology, however, BBB disruption has become a prominent area of investigation. In order to achieve BBB disruption, pulsed exposures at low pressure amplitudes and relatively low frequencies compared to traditional therapeutic ultrasound treatments (~500 kHz vs 1 MHz) are provided immediately following the administration of intravenous ultrasound contrast agents (UCAs). These UCAs are typically lipid or albumin-encased gas microbubbles that are 1–5 microns in diameter and concentrate by the capillary walls, where they undergo oscillation in response to the varying pressure field of the ultrasound wave. The resulting stable (ie, non-inertial) cavitating bubbles exert stresses on the endothelial cell tight junctions, ultimately producing transient disruption of the BBB.

Compromise of the BBB may endure up to 4–6 hours, depending on the molecular weight of the compound being transported (for a review, see Burgess and Hynnen⁶⁶). Microbubbles enhance the effects of FUS, lowering the threshold for cavitation and allowing the acoustic energy to be delivered at non-destructive intensities to the surrounding tissues. Numerous preclinical studies have identified the ultrasound parameters that maximize BBB opening while minimizing tissue damage.^{67–70}

The ability to transiently disrupt the BBB without causing tissue damage has the potential to dramatically alter the landscape of drug delivery to the brain. MRgFUS, in combination with microbubbles, has already been used in preclinical models to enhance the delivery of a number of agents that are typically too large to cross the BBB. This approach was first successfully demonstrated using liposome-encapsulated doxorubicin,^{71–73} with subsequent studies examining intravenous methotrexate,⁷⁴ 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU),⁷⁵ temozolomide,⁷⁶ and the monoclonal antibody trastuzumab (Figure 3).^{77,78} Recently, repeated opening of the BBB was achieved in the 9L rat glioma model using MRgFUS. Three weekly sessions of ultrasound-induced BBB disruption increased the intracerebral concentration of intravenously delivered liposomal doxorubicin, resulting in clinically relevant concentrations and significantly longer median survival ($P < .001$).^{79,80} The safety of these treatments was confirmed by repeatedly disrupting the BBB in the central visual field targets or basal ganglia of non-human primates. BBB opening in visual field targets was successful in 163 of 185 targeted locations, while in the basal ganglia the BBB was successfully opened in 24 of 25 FUS treatments. The animals did not demonstrate any long-term visual or motor deficits.^{81,82}

The abundance of preclinical work demonstrating the safety and efficacy of BBB disruption using FUS has culminated in an ongoing phase I trial (NCT02343991) of MRgFUS with intravenous microbubbles in brain tumor patients who are being treated with doxorubicin. The first patient to be enrolled in the trial was recently treated, and the BBB was successfully opened. Confirmation was obtained via contrast enhancement on the post-sonication MRI, and the patient was subsequently taken to the operating room for surgical resection. Using intraoperative neuronavigation, the surgeon was able to select areas in which the BBB was or was not opened, and analysis of the drug concentrations in the corresponding regions is currently ongoing. Additional patients are being recruited for the study, and once the safety of the technique is established in humans, future studies will further evaluate its efficacy.

Ultrasound-Assisted Local Delivery

Once an agent has overcome the BBB, another critical barrier to achieving the desired therapeutic effect is the tissue/tumor penetration barrier.⁵⁸ This is formed by the anisotropic, electrostatically charged extracellular space (ECS) and gliolymphatic system (GLS) that comprise the space between brain cells.⁸³ The extracellular matrix (ECM), in particular, accounts for 10%–20% of the total brain volume and forms a dense network that limits the dispersion of therapeutic agents, even when they are delivered locally via surgically implanted drug-loaded interstitial wafers or catheter-based convection-enhanced delivery (CED).⁸⁴ The limited distribution of drugs and other therapeutics to areas of the brain that

have been invaded by tumor cells is a major limitation to current treatment. One recent approach being explored involves the engineering of “brain-penetrating” nanoparticles with a dense poly(ethylene glycol) coating that prevents non-specific binding to components of the brain ECM.^{85–87} Ultrasound, however, may produce tissue effects that complement these innovative nanoparticle formulations.

Preclinical work has demonstrated that pFUS can safely produce mechanical effects that alter tissue architecture and improve permeability in tumors outside of the CNS, with significant improvement on the distribution of locally administered agents. In one study, pFUS exposures not only improved the distribution of fluorescent polystyrene nanoparticles in squamous cell carcinoma xenografts, but also enhanced the efficacy of tumor necrosis factor alpha (TNF α) plasmid injections. Electron microscopy revealed gaps, sometimes microns in size, between the tumor cells exposed to pFUS.⁸⁸

Whether similar effects can be produced in brain tissue is currently under investigation. Liu et al⁸⁹ performed a range of in vitro and in vivo studies with pulses of unfocused ultrasound. Using excised porcine brain tissue and orthotopic 9L tumors, they demonstrated that ultrasound increases brain tissue and tumor permeability to variably sized³H-labeled molecules (water, mannitol, inulin, and dextran) increased by 2.3- to 8.8-fold following sonication, with smaller molecules demonstrating larger increases in permeability. Additional in vivo work in a primate model showed that 1 MHz unfocused pulsed ultrasound (applied through a craniotomy) enhanced the distribution of liposomes containing an MR contrast agent following CED.

Lewis et al⁹⁰ expanded on this work, using a transducer cannula assembly to apply low-intensity, continuous, unfocused ultrasound during CED (Figure 4). Sonication increased the volumetric distribution of Evans blue dye (EBD) injected into the caudate nucleus of rats by a factor of 2.24 to 3.25, although, for unclear reasons, adding microbubbles to the infusate reduced the distribution of dye. The authors hypothesized that the microbubbles may have been confined to the tip of the CED catheter; alternatively, they may have attenuated the acoustic energy. A subsequent study used the time-reversal acoustics method to apply focused ultrasound with a custom-designed “smart needle” that incorporated an infusion catheter with a piezo-electric ultrasound transducer.⁹¹ Using this technique, the distribution of EBD increased by a factor of 7.2 in brain-mimicking agarose gels, and by 75% in the caudate nucleus of anesthetized rodents. In this case, microbubble administration slightly improved the distribution of dye. However, further studies are needed to determine the true effects of ultrasound on brain cytoarchitecture in an effort to better understand this phenomenon.

IMMUNOMODULATION

The development of immunotherapy, which involves priming the host’s immune system to recognize a tumor as foreign material, has been an expanding area of GBM research. FUS may indirectly contribute to this process, as evidence has shown that tumor ablation produces cellular debris that contains tumor-specific antigens (TSAs)⁹² and may also enhance tumor-specific T cell activity.^{93,94} In mice with subcutaneous C1300 neuroblastoma

tumors, FUS-induced ablation reduced tumor growth in animals that were re-challenged, presumably due to the immunomodulatory effects of the initial ablation.⁹⁵ The debris produced by FUS has even been used to produce a vaccine to activate the immune system and induce a tumor-specific response in a mouse model of hepatocellular carcinoma.⁹⁶ A number of mechanisms have been proposed. On the one hand, thermal ablation may relieve the immunosuppression caused by the tumor and may upregulate heat shock proteins (HSPs) such as HSP70, an intracellular molecular chaperone that can modulate the immunogenicity of tumors by presenting TSAs to antigen presenting cells (APCs). Indeed, hyperthermia has been implicated as a modulator of gene expression in T-lymphocytes.⁹⁷

However, mounting evidence suggests that it is not the thermal effects at the center of the focal beam, but rather effects that occur at the border of the ablative lesion that play the dominant role. In these regions, intensities are insufficient to generate cytotoxic heat, but still high enough to generate destructive mechanical effects. In an in vitro study, mechanical exposures resulted in the release of HSP60, an endogenous danger signal, by tumor cells, which in turn activated APCs, resulting in increased secretion of TNF- α and interleukin-12 by macrophages and dendritic cells, respectively.⁹⁸ This work was followed by in vivo studies using mice with MC-38 colon adenocarcinoma,⁹⁹ H22 hepatocellular carcinoma,^{96,100} and RM-9 prostate cancer tumors.¹⁰¹ In these studies, pFUS exposures generating primarily mechanical effects resulted in increased infiltration of dendritic cells into the primary tumor, elevated cytotoxic T-lymphocyte activity, increased production of tumor-specific interferon gamma, down-regulation of signal transducer and activator of transcription 3 (STAT3), and slower tumor growth upon re-challenge.

Clinical studies have confirmed an enhanced immune response following FUS treatments in patients. Several groups have documented the preservation of some tumor antigens in the tumor debris following FUS treatment¹⁰² as well as increased levels of natural killer cells and CD4+ T-lymphocytes,¹⁰³ improved CD4+ to CD8+ ratios,^{104,105} and decreased levels of immunosuppressive cytokines such as VEGF and transforming growth factor-(TGF) β 1.¹⁰⁶ Despite mounting preclinical and clinical evidence, studies involving tumors of the central nervous system are still lacking.

CONCLUSION

MRgFUS offers exciting options for the noninvasive treatment of numerous neurological disorders. Within the field of neuro-oncology, the ablative applications of MRgFUS are the closest to being implemented clinically, with several clinical trials already exploring the technology's safety and feasibility in patients. However, thermal ablation is only one of several potential applications of FUS. Other applications currently being explored include SDT, radiosensitization, drug delivery, and immunomodulation. Most of this work has been performed in other organ systems, and further studies of ultrasound's effects in the unique microenvironment of the brain are necessary if we are to fully take advantage of this innovative technology. Additional applications in the future could also include the use of ultrasound to generate low-level, nondestructive heat for deploying therapeutic agents from thermosensitive liposomes or activating heat shock promoters for spatiotemporal control of transgene expression. Pre-clinical reports on these targeted applications have generated

much enthusiasm, and the feasibility for clinical translation is supported by the current state of the technology. As the technology is refined even further, we can expect to see FUS take its place as a valuable adjuvant therapy in the fight against brain cancer.

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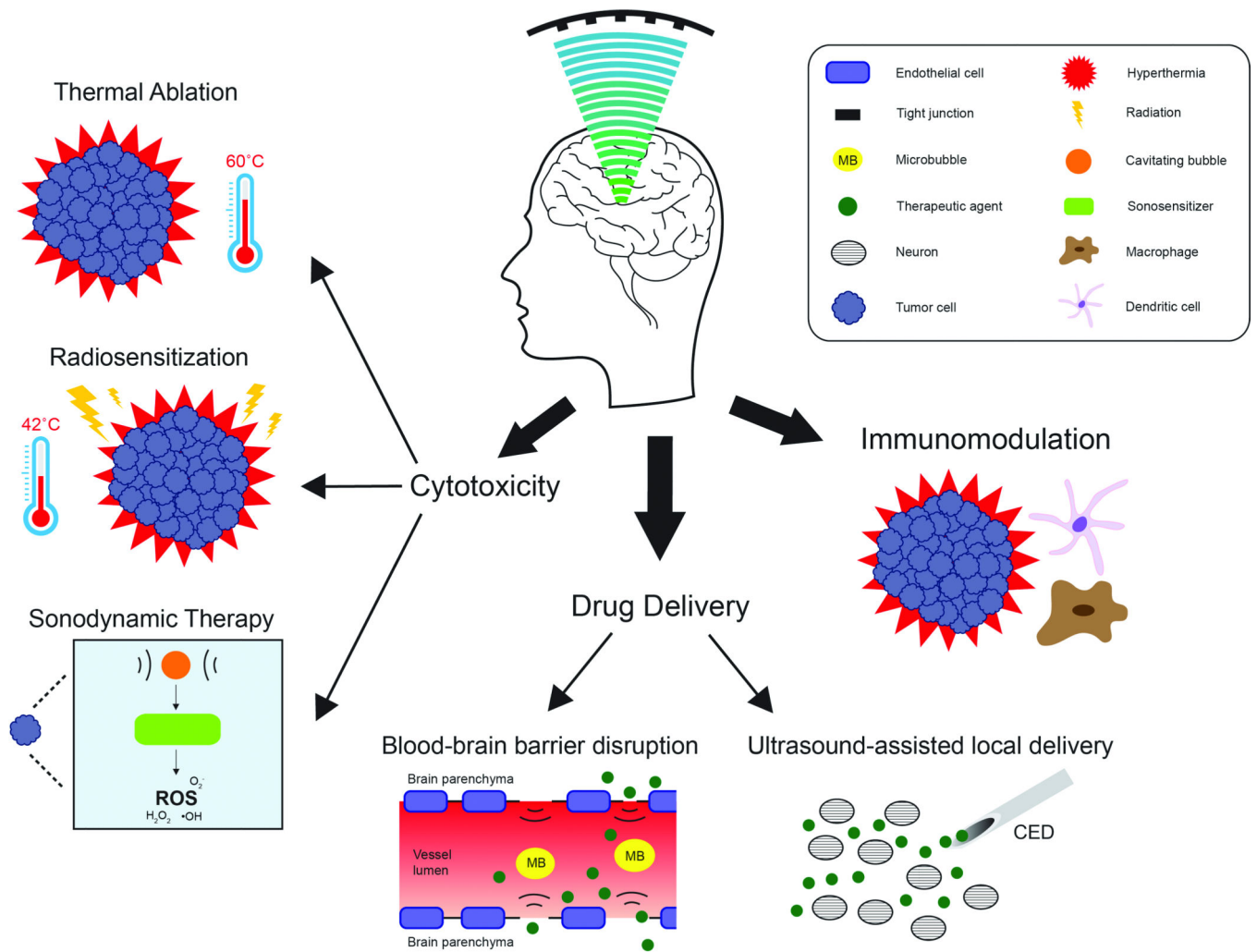


Figure 1. Applications of therapeutic ultrasound in neuro-oncology. Transcranial focused exposures may be used for thermal ablation, radiosensitization, sonodynamic therapy, BBB disruption, ultrasound-assisted local delivery, and immunomodulation. CED, convection-enhanced delivery; ROS, reactive oxygen species.

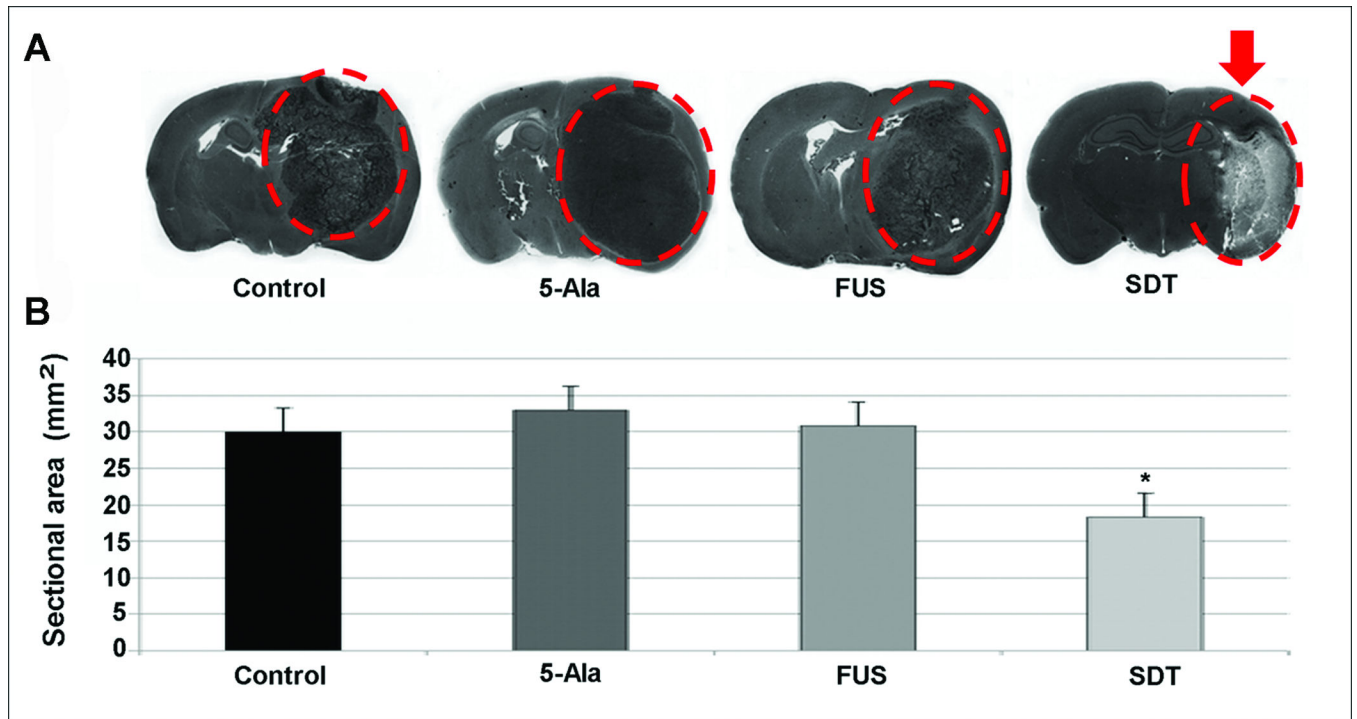


Figure 2. Effect of SDT in a rat C6 intracerebral glioma model. A, coronal brain sections depicting selective destruction of the tumor (outlined by the dashed circle) in the group treated with SDT (arrow). B, tumor size in coronal sections for each treatment group. An asterisk indicates statistical significance ($P < .05$) when compared to rats undergoing a sham operation. 5-Ala, 5-aminolevulinic acid; FUS, focused ultrasound; SDT, sonodynamic therapy. Modified with permission from Ohmura T, Fukushima T, Shibaguchi H, et al. Sonodynamic therapy with 5-aminolevulinic acid and focused ultrasound for deep-seated intracranial glioma in rat. *Anticancer Res.* 2011;31(7):2527–2533.²⁷

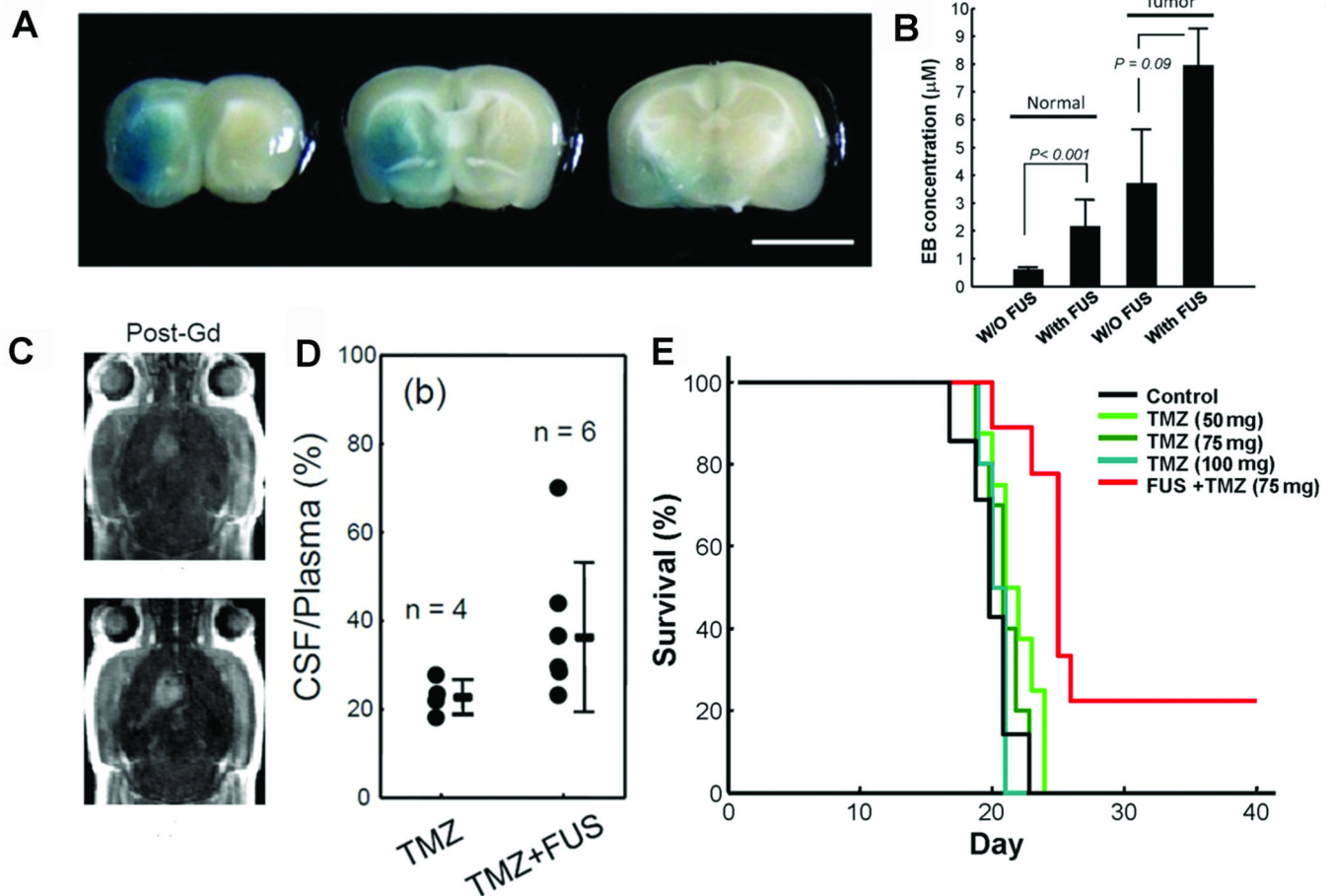


Figure 3.

Ultrasound-mediated opening of the BBB. A, coronal brain sections depicting extravasation of Evans blue dye (EBD) following FUS-mediated opening of the BBB (scale = 5 mm). B, quantification of EBD concentration with or without FUS in normal rats (sonication produced a 3.8-fold increase, $P < .001$) and in rats with tumors (sonication produced a 2.1-fold increase, $P = .09$). C, axial, contrast-enhanced T1-weighted magnetic resonance imaging sequences demonstrating increased contrast-enhancement following sonication of a brain tumor in a rat (lower) relative to a tumor that did not undergo sonication (upper). D, CSF-to-plasma ratio of the TMZ concentration in animals treated with TMZ only or combined TMZ with FUS-BBB opening (TMZ + FUS). E, Kaplan-Meier plot demonstrating improved survival of animals in the TMZ + FUS group compared to control animals or those receiving TMZ of various doses alone. BBB, blood-brain barrier; EBD, Evans blue dye; FUS, focused ultrasound; TMZ, temozolomide. Modified with permission from Wei KC, Chu PC, Wang HY, et al. Focused ultrasound-induced blood-brain barrier opening to enhance temozolomide delivery for glioblastoma treatment: a preclinical study. *PLoS One*. 2013;8(3):e58995.⁷⁶

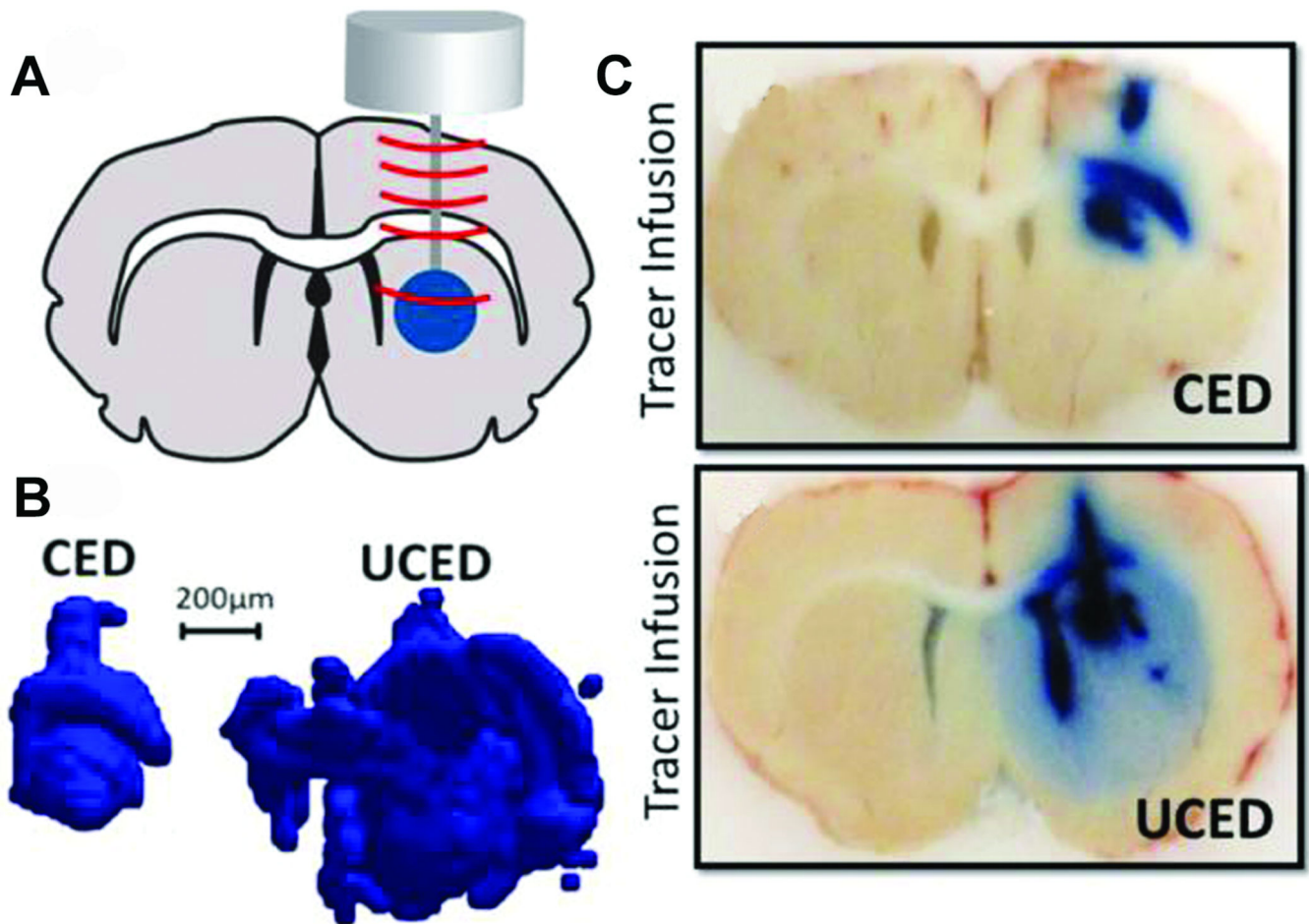


Figure 4. Ultrasound-assisted convection-enhanced delivery (UCED) to the rodent brain. A, a plane wave transducer with a cannula through-hole is mounted on top of the rodent brain through a small craniotomy window in the skull. B, 3-dimensional reconstruction of the distribution of infused EBD with and without ultrasound exposure. C, brain slices in the cannula path showing EBD distribution with (lower) and without (upper) ultrasound exposure. CED, convection-enhanced delivery; EBD, Evans blue dye; UCED, ultrasound-assisted convection-enhanced delivery. Reproduced from Lewis GK Jr., Guarino S, Ghandi G, et al. Time-reversal techniques in ultrasound-assisted convection-enhanced drug delivery to the brain: technology development and in vivo evaluation. *Proc Meet Acoust.* 2011;11:20005–20031,¹⁰⁷ with the permission of the Acoustical Society of America.

Table 1

Applications of focused ultrasound in neuro-oncology

Application	Rate of energy deposition	Energy intensity	Acoustic mechanism	Biological effect
Thermal ablation	Continuous	High	Hyperthermia (high)	Coagulative necrosis
Radiosensitization	Continuous	Low	Hyperthermia (low)	Prevents DNA repair
Sonodynamic therapy	Pulsed	High	Inertial cavitation	Formation of reactive oxygen species
Blood-brain barrier opening	Pulsed	Low	Stable cavitation	Disruption of tight junctions
Enhanced local delivery	Pulsed	Low or High	Radiation forces	Tissue displacement and shear forces
Immunomodulation	Continuous and Pulsed	High	Miscellaneous ^a	Miscellaneous ^a

^aUltrasound-mediated immunomodulation may occur due to thermal or mechanical exposures, with the latter being more prominent. The result is the presentation of tumor-specific antigens to antigen presenting cells, changes in chaperone expression and cytokine secretion.

Table 2

Completed and ongoing clinical trials studying focused ultrasound in neuro-oncology

Application	Phase	Tumor Type	Patients	Adverse Effects	Outcome	Reference
Thermal ablation	I	Recurrent glioblastoma	3	• •	Mild transient worsening of pre-existing dysphasia Thermal lesion in right cerebral peduncle, mild left hemiparesis	Ram et al, 2006 ²²
	I	Glioblastoma	4	•	Intracranial hemorrhage resulting in mortality (n = 1)	McDannold et al, 2010 ³⁵
Radiosensitization	I/II	High grade glioma or brain metastasis	Ongoing	-	-	NCT01698437, NCT00147056, NCT01473485
	I	Grade III astrocytoma and glioblastoma	15	• • • •	Increased deficit (n = 1) Scalp heating (n = 1) Intracranial hemorrhage (n = 4) ^a Superficial thermal lesion (n = 2)	Guthkelch et al, 1991 ²⁴
Sonodynamic therapy	-	-	-	-	-	-
Blood-brain barrier opening	I	Glioblastoma	1	Unknown ^b	Blood-brain barrier opening confirmed with contrast enhancement on MRI ^b	NCT02343991
Enhanced local delivery	-	-	-	-	-	-
Immunomodulation	-	-	-	-	-	-

^aIntracranial hemorrhage occurred as a result of thermocouple probe placement, and was symptomatic in 2 cases.

^bRecruitment of additional patients is currently ongoing. Additional information is needed before comments regarding adverse effects and outcomes can be offered.