Focused ultrasound disruption of the blood-brain barrier: a new frontier for therapeutic delivery in molecular neurooncology

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Recent advances in molecular neurooncology provide unique opportunities for targeted molecular-based therapies. However, the blood-brain barrier (BBB) remains a major limitation to the delivery of tumor-specific therapies directed against aberrant signaling pathways in brain tumors. Given the dismal prognosis of patients with malignant brain tumors, novel strategies that overcome the intrinsic limitations of the BBB are therefore highly desirable. Focused ultrasound BBB disruption is emerging as a novel strategy for enhanced delivery of therapeutic agents into the brain via focal, reversible, and safe BBB disruption. This review examines the potential role and implications of focused ultrasound in molecular neurooncology.

KEY WORDS • brain tumor • blood-brain barrier • focused ultrasound • chemotherapy

MALIGNANT glioma is the most common primary brain tumor in adults. 25 It exhibits extensive invasion into the brain parenchyma, thereby evading the current multimodal therapeutic paradigms of conventional chemotherapy, radiotherapy, and surgery. Despite tremendous advances in this multimodal treatment scheme, the prognosis for patients with malignant glioma remains dismal. 68–70 The mean survival after symptom onset remains 12–16 months, with over 70%–80% of patients dead within 2 years. 69 The highly invasive nature and the complex molecular underpinnings of the disease both account for its intransigence to current conventional therapies. Hence, molecularly directed therapeutic targets are essential to improve clinical outcome. As a result, there is an impetus to deciphering critical signaling pathways in hopes of identifying putative molecular targets.

However, a major challenge to the delivery of molecular pathway–targeting therapeutics is the transport across the BBB, which creates both structural and physiological impediments to delivery. 53 The BBB limits the effectiveness of many conventional chemotherapeutic drugs, making systemic administration an ineffective option for the vast majority of chemotherapy agents. 17 For instance, doxorubicin is a chemotherapy agent that does not appreciably cross the BBB yet has been very effective against malignant gliomas in vitro. 79 Similar delivery challenges plague molecular-based targeted glioma therapy given the various transport mechanisms that govern BBB delivery.

Generally, for therapeutic agents to cross the BBB, they must employ either passive or active transport mechanisms. 51 Small (< 400 D) nonpolar lipophilic agents are easily transported passively, 52 whereas polar or water-based compounds generally require active transport mechanisms. 38, 39 Furthermore, the BBB expresses drug-efflux transporter proteins that physiologically exclude therapeutic agents from the brain. A well-known efflux transporter is the P-glycoprotein, which is a substrate for most chemotherapy agents. 2, 33 Another confounding factor that could significantly affect delivery of therapeutic agents into brain tumors is the potential variability in vascular permeability among tumors, as well as between tumors and normal brain. 3, 5, 6, 10, 16, 46, 47, 55 Potential solutions require either structural modifications of therapeutic agents or transient, safe, and reversible modifications of the BBB to enable delivery into the brain. The latter strategy of BBB modification appears practically more appealing given the complexities of redesigning and modifying molecular therapeutic agents. Ideally, strategies that transiently increase BBB permeability should be focal, safe, reversible, and noninvasive.

Abbreviations used in this paper: BBB = blood-brain barrier; FUS = focused ultrasound; HSV = herpes simplex virus; MNP = magnetic nanoparticle.
Focused ultrasound disruption of the BBB is emerging as a very promising novel noninvasive technology that can circumvent some of the anatomical limitations of the BBB, thereby enhancing delivery of therapeutic agents into the brain. Low-frequency ultrasound waves are delivered transcranially and result in BBB disruptions in focal areas of the brain. Given the potential for focal and selective delivery in conjunction with the inherent advantages of enhanced therapeutic delivery, this review will examine the potential applications and implications of FUS in molecular neurooncology.

**Conventional Nonfocal BBB Disruption Strategies**

Since most therapeutic agents do not readily cross the BBB, several strategies to temporarily disrupt the BBB have been embarked upon previously (Table 1). Transient disruption of the BBB can be obtained using an osmotic agent such as mannitol, which is delivered intraarterially via carotid arteries. The feasibility of such an approach has been demonstrated in brain tumors. Furthermore, alkylated alcohols such as alkyl-glycerol open the BBB in a similar fashion when delivered intraarterially. Besides osmotic agents, there are receptor-mediated mechanisms that can enhance permeability of the BBB. A notable example is the bradykinin system where analogs such as RMP-7 have been employed for enhanced permeability of the BBB. Preclinical application of bradykinin analogs with intraarterial carboplatin appeared promising with increased drug levels in the brain. However, when a similar strategy was applied in Phase II trials for childhood brain tumors and recurrent gliomas, the clinical results were not as promising. A major pitfall was failure to obtain reasonable therapeutic concentrations within the brain.

Enhanced permeability of the BBB can have both beneficial and deleterious effects. Although the BBB limits delivery of therapeutics into the brain, it also protects the brain from systemic toxins. Blood-brain barrier disruption strategies that employ osmotic agents such as mannitol or bradykinin analogs can result in widespread BBB disruption and the potential for deleterious consequences. Therefore, an obvious shortcoming of the aforementioned BBB disruption strategies is the lack of targeted delivery applications.

**Focused Ultrasound Disruption of the BBB: Principles**

A unique advantage of FUS disruption of the BBB over other conventional BBB disruption schemes is the selective and regional permeability increases that result in enhanced local delivery within the brain. The technique entails transcranial delivery of low-frequency ultrasound waves that ultimately result in disruption of the BBB (Fig. 1). Typically, ultrasonic exposure burst at 10 msec with pressure amplitudes less than 1 MPa are conventionally used for durations of 20–30 seconds repeated at the frequency of 1 Hz. By employing low frequencies, the chances of permanent tissue damage are minimized. The technique can be used in conjunction with MR imaging for targeting purposes and documentation of focal BBB disruption, which is manifest by regional contrast extravasation (Fig. 2). Incorporation of intravenously administered lipid-encased perfluorocarbon gas microbubbles (1–5 μm in diameter) further lowers the frequency threshold for BBB disruptions, thereby allowing for much lower and safer frequencies to be used. The feasibility of microbubble-assisted FUS disruption of the BBB was first successfully demonstrated a decade ago. The FUS BBB disruption effects are not as apparent in the absence of microbubbles because acoustic powers are 2 orders of magnitude lower. As the microbubbles traverse the capillaries, they can expand and collapse based on the ultrasonic input. It is hypothesized that FUS results in oscillation and concentration of microbubbles by the capillary walls, which in turn imparts mechanical forces that could result in BBB opening. Furthermore, the microbubbles emit acoustic signals that have been highly correlated with BBB disruption in the absence of vascular damage, thus suggesting that acoustic signals could serve as a surrogate for safety. The safety of FUS disruption of the BBB is well documented, and the overall effects are transient and reversible with no overt neuronal injury. The ensuing BBB disruption lasts at most for approximately 4 hours.

**Focused Ultrasound Disruption of BBB: Design**

The overall schematic for preclinical FUS systems for BBB disruption is illustrated in Fig. 3. The animal is anesthetized and positioned supine with the scalp submerged in a chamber containing degassed water. Low-frequency ultrasound waves emitted from a focused transducer are transmitted through the degassed water into the cranium. Prior to BBB disruption, animals receive lipid microbubbles and the therapeutic agent of interest. Magnetic resonance imaging of the brain is often used to select a focal area of interest for disruption of the BBB. Disruption of the BBB is then accomplished using a burst of low-frequency ultrasound. Magnetic resonance imaging of the animal brain is performed prior to and after FUS BBB disruption. The region of BBB disruption

### TABLE 1: Enhanced BBB delivery strategies

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<th>Strategy</th>
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<tr>
<td>intraarterial via carotid arteries</td>
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<td>intraventricular</td>
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<tr>
<td>osmotic agents such as mannitol</td>
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<td>BBB permeation analogues such as bradykinin</td>
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<td>convection-enhanced interstitial delivery</td>
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### TABLE 2: Advantages of FUS-mediated therapeutic delivery

<table>
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<th>Advantage</th>
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<tr>
<td>focal &amp; targeted delivery minimizes problems seen w/ widespread BBB disruption</td>
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<tr>
<td>transient disruption of BBB, hence reversible</td>
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<tr>
<td>noninvasive transcranial technique</td>
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<tr>
<td>enhanced delivery of chemotherapy, gene therapy, &amp; monoclonal antibodies across BBB</td>
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is confirmed on T1-weighted contrast-enhanced MR images (Fig. 2).

**Preclinical Applications of FUS**

Table 3 provides a succinct summary of enhanced BBB delivery strategies.

**Delivery of Antibodies Into the Brain**

One of the most practical therapeutic attributes of FUS is the ability to deliver antibodies into the brain, which was demonstrated in a previous study. Kinoshita et al. were able to demonstrate the BBB crossing of dopamine D(4) receptor–targeting antibody and subsequent antigen recognition within the brain via FUS mediation. Traditionally, a major hurdle in antibody therapeutics is the very limited ability of antibodies to cross the BBB in light of absent large water channels or active transport mechanisms for antibodies in cerebrovascular endothelium. However, there are several neurological disorders for which antibody-mediated therapy would be advantageous. For instance, in Alzheimer disease, there is accumulation of amyloid-β plaques, which form the basis for toxicity and cognitive impairment. Interestingly, direct intracranial administration of anti-amyloid β antibodies in transgenic mice, as well as in normal animals, led to a substantial amount of plaque reduction. A hindrance of direct intracranial injections is its invasiveness. Most recently, using MR imaging–guided FUS, anti-amyloid β antibodies were intravenously delivered into transgenic mice, and this resulted in significant plaque reduction 4 days posttreatment. The investigators also noted that with FUS, a much lower dose of antibodies was effective as well for plaque reduction.

Another potential area of interest pertaining to antibody therapy is cancer targeting. Antitumor monoclonal antibodies that have been successfully used to target systemic cancers could have potential applications for metastatic or primary cancers in the brain. For instance, Herceptin, which is an anti-HER2 monoclonal antibody and a therapeutic target of breast cancer, was successfully delivered across the BBB with MR imaging–guided FUS as a proof-of-concept. This proof-of-concept is very encouraging in light of potential extrapolations to other antitumor antibody candidates.

**Delivery of Conventional Chemotherapy Agents Into the Brain**

Given that the BBB significantly compromises the bioavailability of conventional chemotherapy agents into the brain, several strategies have been previously employed. The preclinical results have been promising, but only minimal benefits have been documented in clinical trials. One approach of augmenting brain concentration of chemotherapy agents has been the use of biodegradable matrices. Matrices laden with chemotherapy agent are implanted within the tumor resection cavity. Other approaches have included convection-enhanced delivery systems and intraarterial chemotherapy. However, even with these approaches, insufficient chemotherapy bioavailability renders their efficacy suboptimal.

As a result, preclinical studies have been undertaken with FUS to assess the feasibility of chemotherapy delivery into brain tumors. This strategy was first successfully applied using doxorubicin, which does not appreciably cross the BBB. The investigators successfully demonstrated a substantial increase in the concentration of liposome-encapsulated doxorubicin within the FUS-treated hemispheres of normal rats compared with the nontreated hemisphere. In a subsequent follow-up study, the investigators demonstrated a substantial therapeutic benefit...
from FUS-mediated delivery of liposome-encapsulated doxorubicin in rats with intracranial gliomas.75

The delivery of methotrexate into the brain using FUS disruption of the BBB has been investigated as well. Mei and colleagues43 assessed the FUS-assisted intracranial delivery of intravenous methotrexate into rabbit brains and compared this method with an intra–carotid artery injection. They noted targeted and enhanced delivery of intravenous methotrexate up to 10-fold with FUS. Furthermore, the FUS delivery strategy was noted to be more effective than intra–carotid artery delivery alone.

Most recently, Liu and colleagues36 investigated FUS-mediated delivery of BCNU chemotherapy in animals with brain tumors. They reported substantial increases in tumor BCNU bioavailability in animals treated with FUS compared with nontreated animals. Accordingly, tumor progression was significantly compromised as evident by decreases in tumor size. The authors further demonstrated a significant survival benefit associated with FUS-mediated delivery of BCNU.

The preclinical data appear promising to date for FUS-mediated delivery of chemotherapy agents into the brain. Preliminary results demonstrate both feasibility and efficacy. Additional successful preclinical applications should pave the way for clinical studies.

**Delivery of Therapeutic Nanoparticles Into the Brain**

Nanotechnology-based delivery systems have generated substantial interest in light of demonstrated tumor-targeting applications.28 Through such unique features, nanoparticle-delivery platforms could potentially circumvent some of the challenges associated with conventional chemotherapy for malignant brain tumors. However, the BBB remains a critical limitation for nanoparticle. For instance, gold nanoparticles have gained prominence in nanotechnology-mediated cancer targeting for systemic cancers,7,10,50 yet gold nanoparticles are significantly limited in their biodistribution to the brain following systemic delivery.11,66,72 Therefore, strategies such as FUS that can increase BBB permeability to nanocarriers could enhance applicability of nanotechnology-based targeting in malignant brain tumors.

Liu and colleagues37 recently assessed FUS-mediated delivery of an iron oxide MNPs conjugated to an antineoplastic agent, epirubicin. They used MNPs because of the favorable MR imaging characteristics, which could facilitate imaging. They demonstrated a substantial accumulation of MNPs, as well as epirubicin, up to 15 times the therapeutic range in the brain when delivered with FUS. They further showed decreased tumor progression in animals with brain tumors that received MNP with epirubicin via FUS. Similar to intracranial MNP targeted delivery, we have recently observed targeted enhanced parenchymal delivery of polyethylene glycol–coated gold nanoparticles into normal brain and to the periphery of parenchymal brain tumors in a rat model (AB Etame et al., unpublished data, 2011).

The prospects of combining FUS with nanoparticle-delivery platforms create a whole new avenue for targeting opportunities. The nanoparticle platforms are already very attractive for molecular targeting because nanoparticles can be easily functionalized with small molecule inhibitors, proteins, nucleic acid, ligands, and antibodies in any combination onto the surface of the nanoparticle.
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Delivery of Gene-Based Therapies Into Brain Tumors

One of the major challenges with viral-based gene therapy for malignant brain tumors has been the inadequacy of therapeutic delivery. Delivery strategies have included intratumoral injection, convection-enhanced delivery systems, and intraventricular delivery. However, all 3 modalities are invasive. In addition, there are toxicity-related issues especially with intraventricular delivery. On the other hand, intravascular delivery has been shown to be safe, but it is plagued by BBB limitations, which can be alleviated with BBB disrupting agents. Because glioma gene therapy strategies are geared toward targeting invasive tumor cells that might have escaped local therapies of surgery and radiosurgery, focal and targeted delivery is very essential. Hence, FUS could play a major role in this endeavor. The main preclinical assessment of FUS-mediated delivery of viral vectors into the brain was performed using radiolabeled HSV vector. Using a combination of autography and histological assessments, the investigators were able to demonstrate focal delivery of intravenously administered HSV within the hemisphere that was sonicated with FUS. While this study is encouraging, additional studies are warranted to assess the functional efficiency of this mode of viral vector delivery into the brain. Transgene expression via FUS

**TABLE 3: Preclinical animal studies of FUS delivery across the BBB**

<table>
<thead>
<tr>
<th>Authors &amp; Year</th>
<th>Study Finding</th>
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<tr>
<td>Kinoshita et al., 2006</td>
<td>delivery of dopamine D(4) receptor–targeting antibody into brain</td>
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<tr>
<td>Sheikov et al., 2006</td>
<td>focal delivery of intravenously administered HSV-engineered vector across BBB of normal rats</td>
</tr>
<tr>
<td>Kinoshita et al., 2006</td>
<td>delivery of Herceptin (anti-HER2 monoclonal antibody) into brain</td>
</tr>
<tr>
<td>Treat et al., 2007</td>
<td>successful enhanced delivery of doxorubicin across BBB of normal rats</td>
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<tr>
<td>Treat et al., 2009</td>
<td>successful enhanced delivery of doxorubicin across BBB of rat glioma models</td>
</tr>
<tr>
<td>Mei et al., 2009</td>
<td>successful enhanced delivery of doxorubicin across BBB of rabbit brain</td>
</tr>
<tr>
<td>Liu et al., 2010</td>
<td>enhanced delivery of BCNU w/ FUS led to tumor remission &amp; prolonged survival in rat glioma model</td>
</tr>
<tr>
<td>Jordão et al., 2010</td>
<td>delivery of anti–amyloid β antibodies via FUS led to Alzheimer plaque reduction in transgenic mouse model</td>
</tr>
<tr>
<td>Liu et al., 2010</td>
<td>FUS delivery of iron oxide MNPs conjugated to epirubicin led to enhanced delivery, tumor remission, &amp; prolonged survival in rat glioma models</td>
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delivery of DNA constructs could serve as an alternative to viral intracranial delivery methods.\(^{6,17,11}\) Focused ultrasound has been previously employed for intraterine gene delivery into fetal brain.\(^{14}\) Furthermore, Shimamura and colleagues\(^{65}\) have demonstrated enhanced delivery and expression of the luciferase gene in rat CNS using the microbubble-enhanced ultrasound method. The use of FUS-mediated targeted molecular therapy can thus be envisioned. For example, this technology could be used to deliver a gene that could complement a mutation in a tumor cell or, by delivery of a silencing gene, such as microRNA or short hairpin RNA gene, to downregulate the expression of an aberrantly expressed protein. Reduc-
tion in the expression of Survivin, an inhibitor of apoptosis, which is expressed in malignant glioma, has been achieved in a flank xenograft cervical cancer model using focused ultrasound delivery of a short hairpin RNA silencing vector targeting Survivin.\(^{9}\) Further preclinical investigation to assess the potential of transcranial focused ultrasound for nonviral gene therapy in the CNS is warranted.

**Future Directions**

Preclinical studies thus far suggest that FUS with microbubbles can safely facilitate the focal delivery of a wide range of therapeutic agents into the brain. In this regard, FUS affords selective and targeted delivery. In addition, the potential to successfully combine FUS with other specialized targeted delivery systems such as nanoparticles and viral vectors carries tremendous promise in molecular neurooncology. Furthermore, FUS applications in animal brain tumor models demonstrate correlations between enhanced delivery and decreased tumor progression with a concomitant improved survival. Safety profiles have equally been established with FUS-mediated delivery strategies from a preclinical standpoint. If these preliminary preclinical results are sustainable, FUS-mediated delivery will play a pivotal role in the molecular therapeutics of malignant brain tumors.

**Disclosure**

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Author contributions to the study and manuscript preparation include the following: Concept and design: Rutka, Etame, Hynynen. Acquisition of data: Etame, Diaz, Smith. Analysis and interpretation of data: Rutka, Etame, Diaz, Hynynen. Drafting the article: Etame. Critically revising the article: Rutka, Diaz, Smith, Mainprize, Hynynen. Reviewed submitted version of manuscript: all authors.

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