Enhanced therapeutic agent delivery through magnetic resonance imaging–monitored focused ultrasound blood-brain barrier disruption for brain tumor treatment: an overview of the current preclinical status

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Malignant glioma is a severe primary CNS cancer with a high recurrence and mortality rate. The current strategy of surgical debulking combined with radiation therapy or chemotherapy does not provide good prognosis, tumor progression control, or improved patient survival. The blood-brain barrier (BBB) acts as a major obstacle to chemotherapeutic treatment of brain tumors by severely restricting drug delivery into the brain. Because of their high toxicity, chemotherapeutic drugs cannot be administered at sufficient concentrations by conventional delivery methods to significantly improve long-term survival of patients with brain tumors. Temporal disruption of the BBB by microbubble-enhanced focused ultrasound (FUS) exposure can increase CNS-blood permeability, providing a promising new direction to increase the concentration of therapeutic agents in the brain tumor and improve disease control. Under the guidance and monitoring of MR imaging, a brain drug-delivery platform can be developed to control and monitor therapeutic agent distribution and kinetics. The success of FUS BBB disruption in delivering a variety of therapeutic molecules into brain tumors has recently been demonstrated in an animal model. In this paper the authors review a number of critical studies that have demonstrated successful outcomes, including enhancement of the delivery of traditional clinically used chemotherapeutic agents or application of novel nanocarrier designs for actively transporting drugs or extending drug half-lives to significantly improve treatment efficacy in preclinical animal models.

KeY WorDs • focused ultrasound • brain tumor • blood-brain barrier • nanocarrier • magnetic resonance imaging

MALIGNANT glioma is a common and severe primary brain tumor with a high recurrence rate and an extremely high mortality rate within 2 years of diagnosis, even when surgical, radiological, and chemotherapeutic interventions are applied. Chemotherapy is currently an important adjuvant treatment that usually follows tumor debulking surgery. However, the efficacy of intravenously administered chemotherapeutic drugs is limited by their adverse systemic effects and poor penetration across the BBB, resulting in both insufficient local drug concentration in the tumor and extensive circulating body toxicity. Focused ultrasound is a recently discovered noninvasive technique that shows great promise for local and reversible enhancement of the permeability of the BBB to chemotherapeutic agents. The efficacy of brain tumor chemotherapy could potentially be significantly enhanced by FUS BBB disruption, as guided and monitored by MR imaging. In this paper we review the current status of brain tumor treatment, the role of the BBB, and current clinical and preclinical treatment modalities. We then describe the current status of MR imaging-monitored FUS BBB disruption, including a review of the technique itself and its application to chemotherapeutic agent delivery. Finally, we describe the novel use of MNPs to concurrently function as MR imaging contrast agents and active magnetic targeting agents for brain tumor treatment, which were developed by our research team.

Abbreviations used in this paper: BBB = blood-brain barrier; BCNU = 1,3-bis(2-chloroethyl)-1-nitrosourea; EPEG = o-(2-aminoethyl)polyethylene glycol; FUS = focused ultrasound; IC50 = half-maximal inhibitory concentration; MNP = magnetic nanoparticles.
Current Status of Brain Tumors and the BBB

At least 18,000 patients are diagnosed with malignant primary brain tumors in the US each year, and more than half of them have glioblastoma. The median survival of patients with low-grade gliomas is 5–15 years, but it is only 9–12 months for patients with high-grade gliomas.

Multicenter randomized trials suggest that the treatment of glioblastoma patients with debulking surgery and radiation therapy results in a median survival of only 12 months. Chemotherapy is also an important treatment modality for glioblastoma, but generally results in a limited and temporary response, while producing side effects that further reduce the quality of the patient’s remaining life.

In the US, the most common systemically administered adjuvant chemotherapeutic drugs are BCNU, procarbazine, vincristine and lomustine, or temozolomide. No particular drug or multidrug regimen with a proven superiority in glioblastoma treatment has emerged, and chemotherapeutic delivery has met with limited success. For example, BCNU has been used since the 1970s and remains a common and effective chemotherapeutic agent for brain tumors, yet it provides only a small benefit in short-term survival. Because of the substantial toxicity of chemotherapeutic agents like BCNU, an important current question is how to deliver a sufficiently high therapeutic dose specifically to the target tumor area to improve performance.

The Blood-Brain Barrier

The BBB consists of the cerebral capillary endothelium, choroid plexus epithelium, and arachnoid membrane. Layered cells in these structures form “tight junctions” containing several proteins. Transcellular transport is further limited by low endocytic activity and the absence of fenestrations. The BBB prevents diffusion of toxic foreign substances into the brain parenchyma, but also presents an almost impenetrable barrier to therapeutics such as cisplatin (molecular weight 299 D). The enhanced permeability and retention effect of therapeutic nanoparticles is also greatly restricted by the BBB.

The concentrations of anticancer drugs in the brain appear to be further limited by the pumping action of P-glycoprotein, a large (140–170 kD) glycosylated transport protein found in the luminal membrane of endothelial cell walls of the BBB. Up to 50% of total glioma cells preserve P-glycoprotein function. Despite the generally leaky nature of the vasculature of gliomas, these new vessels thus maintain some BBB properties that contribute to inefficient drug delivery. Moreover, BBB disruption in these tumors is highly heterogeneous, and the tumor core is often the most permeable compared with the impermeable proliferating brain tumor peripheral region. Permeability does not necessarily correlate with tumor histology, size, or anatomical location, and glioblastoma cells have been found at great distances from the enhancing regions of the tumors.

Current Chemotherapy Route for Brain Tumors

Chemotherapeutics currently delivered by intravenous injection need to reach the brain by penetrating through the BBB or blood-CSF barrier. Some chemotherapeutic agents such as temozolomide are used in both intravenous and oral form. Chemotherapeutic agents can also be delivered interstitially by local injection or by direct implantation of drug-carrying biodegradable matrices into the debulked tumor cavity. A 30-month trial in 240 patients found median survival times of 13.9 and 11.6 months for patients implanted with BCNU wafers or placebos, respectively. However, Gliadel wafer (Eisai) implantation was found to cause adverse effects, including CSF leakage, intracranial hypertension, seizures, brain edema, healing abnormalities, and intracranial infection.

In convection-enhanced delivery, chemotherapeutic agents are interstitially infused while maintaining a pressure gradient, thus generating bulk interstitial fluid flow through the brain after an open-skull procedure. In small animal brains, convection-enhanced delivery has achieved much higher local levels of chemotherapy than intravenous administration. Local drug distribution depends on the volume and rate of the gradient of infusion and on the molecular weight, concentration, and polarity of the drug. Current obstacles to convection-enhanced delivery include low rates and volumes of infusion that can lead to heterogeneous distribution and high variable tumor interstitial fluid pressure that causes a fast efflux of chemotherapeutic drugs from the injection site.

Focused Ultrasound-Induced BBB Disruption

Concepts of FUS BBB Disruption

Recent studies have shown that in the presence of microbubbles and in the low-energy burst-tone mode, FUS can increase the local permeability of the BBB. This BBB disruptive effect is temporary and reversible and does not damage neural cells. Compared with alternative approaches such as modified lipophilic chemicals or hypertonic solutions, clinical trials of these agents showed an increased patient survival from 11.4 to 17.5 months. However, osmotic solutions cause systemic rather than localized alteration of the BBB and have been associated with complications such as stroke-like syndrome, transient (2–3 days) exacerbation of pre-existing neurological deficits, temporary seizures, and potential tumor migration and formation of new tumor nodules at distant brain locations.

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Focused ultrasound BBB disruption for therapeutic agent delivery

The first promising outcome in FUS-enhanced brain drug delivery was the successful enhanced delivery of doxorubicin for preclinical brain tumor treatment.\textsuperscript{58,59} Doxorubicin (Doxil, Ben Venue Laboratories) is a chemotherapeutic agent that can be encapsulated in long-circulating pegylated liposomes. In normal rat brain, Treat et al.\textsuperscript{58} reported that at a microbubble concentration of 0.1 ml/kg, doxorubicin was delivered to the brain parenchyma at a concentration of 886 ± 327 ng/g tissue, thus reaching the therapeutic dose of doxorubicin treatment for breast carcinoma. With higher microbubble concentrations of 0.2 and 0.5 ml/kg, doxorubicin concentrations reached 2369 ± 946 and 5366 ± 659 ng/g tissue in the sonicated area, respectively, whereas doxorubicin concentrations in control tissue samples remained at or below 251 ± 119 ng/g tissue at all levels of the ultrasound contrast agent. The delivered doxorubicin concentration was highly correlated with the microbubble dose, which has since been shown to correlate with brain tissue damage mainly through microhemorrhages. The doxorubicin concentration also showed a strong correlation (r = 0.87) with MR imaging signal enhancement.

Later, Treat et al.\textsuperscript{59} also reported the treatment efficacy of FUS-enhanced doxorubicin delivery in a small-animal glioma model. Following the successful delivery of doxorubicin, FUS-enhanced delivery of BCNU was also attempted.\textsuperscript{37} The lipophilic BCNU (Fig. 2A) is a widely used, effective, and currently clinically approved chemotherapeutic agent for brain tumors.\textsuperscript{17} It has been studied since 1970 and is one of the oldest drugs used to treat glioblastoma. Although BCNU contains lipophilic characteristics that allow its natural form to penetrate the BBB, its treatment efficacy is severely hampered by insufficient concentrations within the tumor due to its substantial body toxicity. Clinical trials demonstrated a modest (10%) benefit in survival over 1–2 years (10.1% at 1 year and 8.6% at 2 years compared with radiation therapy alone), but no significant difference in median patient survival (12 vs 9.4 months for radiation alone\textsuperscript{16}). In contrast, a recent temozolomide Phase III trial showed a statistically significant benefit for patient survival rate (2-year survival rate of 26.5% compared with radiation alone\textsuperscript{26}), with median survival extended to 15 months.\textsuperscript{16}

In a test of enhanced delivery of BCNU in normal animal (rat) brain using an in-house–designed MR imaging-monitored FUS platform (Fig. 1), we reported that the BCNU concentration can be increased up to 340% (from 150 to 513 μg) compared with the contralateral unsonicated brain. When treating the tumor-bearing animal brain, there was a nearly 2-fold increase in BCNU (from 170 to 344 μg) at the tumor. Without presonication, the BCNU...
concentrations at healthy and tumor-bearing brain sites were found to be similar (170 vs 150 μg, respectively).

Treatment efficacy was examined by observing tumor progression. Tumors in the control group grew from $0.13 \pm 0.13 \text{ cm}^3$ (Day 10) to $0.28 \pm 0.11 \text{ cm}^3$ (Day 31), and a similar trend was observed in the FUS-only group (from $0.11 \pm 0.02 \text{ cm}^3$ at Day 10 to $0.32 \pm 0.07 \text{ cm}^3$ at Day 31). The group treated with BCNU alone demonstrated a temporal decrease in tumor progression, but this effect was not sustainable, as tumors eventually grew to a similar level as in the control group ($0.07 \pm 0.03 \text{ cm}^3$ at Day 10 to $0.27 \pm 0.17 \text{ cm}^3$ at Day 31). Combined FUS and BCNU provided the most significant suppression of tumor progression, with tumor size at $0.04 \pm 0.08 \text{ cm}^3$ on Day 31.

When comparing survival, the median survival of control, FUS-only, and BCNU-only groups was 28.5, 25.5, and 33 days, respectively, confirming that BCNU prolonged animal survival. However, treatment with both FUS and BCNU produced the most significant benefit, increasing the median survival to 53 days (2 of 6 in the group survived for more than 60 days). Overall, the results suggest that the use of FUS to enhance BCNU delivery into brain tumors has a superior effect on suppressing tumor progression and improving animal survival than the use of either treatment alone.

**Focused Ultrasound BBB Disruption to Enhance Delivery of Chemotherapeutic Agents for Brain Tumor Treatment**

The successful delivery of clinically approved chemotherapeutic agents into the brain through FUS was reviewed in the previous section. Here we review the current literature of delivering nanoparticles into the brain, as well as our previous work on FUS-enhanced delivery of therapeutic nanocarriers (mainly MNPs), with the intention of improving the efficacy of brain tumor chemotherapy.

**Characteristics of Delivered Substances for BBB Permeability**

Central nervous system diseases such as brain gliomas and Alzheimer disease represent the largest and fastest-growing area of unmet medical needs. Their treatment is severely hampered by the restrictive tight junctions of the capillary endothelial cells of the BBB that limit the pen-
tion of 98% of small molecules and almost all large molecules into the brain tissue.45 Only drugs with low molecular weight and high lipid solubility are capable of crossing this specialized system.1 Developing approaches such as nanobiotechnology to effectively, safely, and conveniently deliver therapeutic drugs to the CNS is therefore critically important.

The ability of nanomedicines to cross the BBB is affected by a number of factors including their size, charge, and surface properties. Nanoparticles must be large enough (30–100 nm) to avoid leakage into capillaries, but not so large (>100 nm) that they are susceptible to macrophage-based clearance. Many studies have focused on the optimum size for nanocarriers used in brain disease treatment. There is general agreement that the volume of the distribution of nanocarriers in rat striatum is inversely proportional to the particle size. MacKay et al.46 demonstrated that the ideal nanoparticle size for drug delivery in brain is less than 100 nm, because above this size, nanoparticles are retained near the site of injection and show restricted mobility. Studies on brain extracellular space yielded more precise information: the extracellular space is estimated to be between 35 and 64 nm in diameter in normal rat brain,53 which means that many vectors larger than 100 nm will be too big to transit the normal neocortical extracellular space. Hydrophilic nanoparticles smaller than 100 nm have also been reported to avoid opsonization2 and consequently show prolonged durations of action, as well as enhanced targeting to specific sites.3 Sonavane et al.52 evaluated the effect of increasing particle size on the biological distribution of nanoparticles following intravenous administration of the nanoparticle suspension prepared in sodium alginate solution in mice. Injection of different-sized gold nanoparticles (15, 50, 100, and 200 nm) clearly revealed that the distribution depends on the particle size in various tissues and organs. A higher amount of 15-nm nanoparticles was observed in all tissues including blood, liver, lung, spleen, kidney, brain, heart, and stomach. Nanoparticles larger than 200 nm were present at the lowest level in the brain compared with 15- and 50-nm particles, which could easily cross the BBB.52

The effect of surface charge on diffusivity of nanoparticles has also been studied in the rat brain. MacKay et al.40 observed that the distribution nanoparticles with modest amounts of positive charge was significantly decreased compared with neutral nanoparticles (p < 0.0005). Cationic liposomes were found adjacent to the needle tract because of nonspecific binding to negatively charged structures in the brain parenchyma.41 The low diffusivity of cationic liposomes presents a challenge because they are used as vectors for gene delivery.19 Based on these results, a neutral or negative surface charge is required to obtain good diffusion. Most nanoparticles have been found to have increased permeability through brain capillary endothelial cells of the BBB (by transcytosis) when vectorized with cationic bovine serum albumin and poly(ethylene glycol)–poly(lactide).39 Positive charges of the chitosan cationic compound may also electrostatically interact with the negatively charged brain endothelium to trigger adsorptive-mediated transport across the BBB.

Surface properties have a considerable impact on the diffusivity of colloidal vectors, especially because of the presence of steric coatings. Polyethylene glycol and dextran coatings significantly increase the distribution of nanoparticles.40 Such biocompatible polymers are known to extend the systemic circulation of nanocarriers because they significantly reduce interactions with proteins.45 Polyethylene glycol has also been shown to increase the BBB permeability of several conjugates.3,65 Nevertheless, when nanoparticles were covered simply by albumin, the effect of size was reduced. The albumin coating masks hydrophobic structures of the polystyrene nanoparticles, thereby reducing the risk of eventual aggregation and binding to proteins in the extracellular space. Tosi et al.66 conjugated poly(ε-lactide-coglycolide) to a similipioid glycopeptide (g7) to produce nanoparticles (g7-nanoparticles) capable of crossing the BBB and delivering several kinds of molecules that are normally unable to cross the BBB to the CNS. In addition, solid lipid nanoparticles have been investigated as drug carriers to cross the BBB. Solid lipid nanoparticles improve the lipophilicity of the drug complex, thereby increasing the chance of transport of the incorporated drug.61 Taken together, permeability of nanoparticles across the BBB requires either higher lipid solubility or surface conjugation of specific targeted ligands to polyethylene glycol derivatives.

Basic Concepts of MNPs for Biological Use

Magnetic nanoparticles are constitutes of magnetite (Fe3O4) that have received high attention for biomedical applications. Magnetic nanoparticles are also known as superparamagnetic iron oxide particles and have been used as contrast agents for MR imaging for more than 20 years.52,65 At the same time, therapeutic applications of MNPs have also rapidly expanded. Magnetic nanoparticles can be conjugated to therapeutic agents such as drugs, proteins, enzymes, antibodies, or nucleotides and directed to specific organs, tissues, or tumors using an external magnetic field. This magnetic targeting is a promising strategy for achieving localized drug delivery to tumor tissue. The deposition, accumulation, and retention of drug-conjugated MNPs in tumors are enhanced by magnetic force. The feasibility of this application has recently been demonstrated in brain tumors.9,11 Chertok et al.9 showed that accumulation of nanoparticles was consistently enhanced with 9.6-fold selectivity for MNP accumulation in gliomas compared with the contralateral brain site. Magnetic nanoparticle distribution can be monitored in vivo by MR imaging in the brain. For example, MR spin-spin (R2) relaxivity measurement has been used to compare the R2 maps of animals that only received intravenously administered MNPs to those of animals that received intravenously administered MNPs combined with 0.4-T magnetic targeting. Image analysis confirmed that MNP distribution could be visualized in vivo in the brain and that magnetic targeting induced a 5-fold increase in MNP accumulation in the total glioma tumor mass.

Dose-Dependent Therapeutic MNPs: In Vitro Considerations

Traditional chemotherapeutic agents can be conjugat-
ed to MNPs to form therapeutic MNPs, for example MNP-epirubicin or MNP-BCNU (Fig. 2A). The concentrations of therapeutic MNPs that were required for 50% inhibition of cellular growth of glioma cells were initially determined in vitro. Pure MNPs without conjugated anticancer drugs have no apparent cytotoxic effect when cocultured in vitro with tumor cells. In contrast, abundant MNP-epirubicin that had presumably been taken up by endocytosis could be observed within cells by transmission electron microscopy. Furthermore, the particles passed into the nuclei and appeared to have induced apoptosis.

Conjugating epirubicin to MNPs did not affect its anticancer ability: the IC_{50} of free epirubicin and MNP-epirubicin was 6.1 and 4.6 µg/ml, respectively. The IC_{50} was reduced significantly to 1.3 µg/ml with magnetic targeting (Fig. 2B). Free-BCNU and MNP-BCNU were also both toxic to C6 cells in a dose-dependent manner. The IC_{50} of MNP-BCNU was 6.9 µg/ml, which is lower than that of free-BCNU (8.6 µg/ml; Fig. 2C) due to greater thermal stability and a decreased rate of hydrolysis of conjugated BCNU, all leading to more efficient delivery of BCNU into the cells at 37°C. Magnetic targeting of MNP-BCNU led to a significant reduction in the IC_{50} to only 4.3 µg/ml (Fig. 2C), suggesting that more of the MNP-BCNU was effectively guided to and concentrated at the target area.

To provide more effective MNPs, a self-protecting high-magnetic nanomedicine (EPEG-MNP-BCNU) was designed by grafting EPEG onto the surface of MNP-BCNU (Fig. 2A). This nanomedicine (EPEG) acts to protect BCNU by slowing down its hydrolysis rate. The half-life of BCNU was thereby prolonged from 30 hours (MNP-BCNU) to 62 hours. Free-BCNU and EPEG-MNP-BCNU were both toxic to U87 cells in a concentration-dependent manner. However, the IC_{50} of the EPEG-MNP-BCNU was 6.4 µg/ml, which was lower than that of free-BCNU (8.5 µg/ml; Fig. 2D). Moreover, the IC_{50} was reduced significantly to only 4.0 µg/ml when an external magnetic field of 800 gauss was applied to the EPEG-MNP-BCNU (Fig. 2D).

**Focused Ultrasound BBB Disruption to Deliver MNPs Into Brain**

Liu et al. first demonstrated the application of FUS BBB disruption to enhance MNP delivery into the brain in small animals (Fig. 3). Their aim was to deliver MNPs into the brain and then use MR imaging monitoring of these MNPs to simultaneously detect BBB disruption and follow the status change of sonicated brain over time. An MNP contrast agent that was clinically approved for blood-pool MR imaging (Resovist, Schering AG Inc.; carboydextran-coated, 60-nm hydrodynamic size) was used. The local distribution of MNPs in the brain causes field inhomogeneity and concomitant signal loss on T2*-weighted images. The T2*-weighted images obtained before and after MNP administration and FUS delivery could therefore be used to detect the BBB disruption effect. This MR imaging-based method to detect BBB disruption was histologically confirmed.

Different levels of FUS pressure were tested, and the signal loss caused by intracranial hemorrhage or MNP leakage could be successfully distinguished by obtaining 2 T2*-weighted images before and after intravenous administration of MNPs. The biodistribution of MNPs in the brain could also be followed over time by collection of T2*-weighted images. Over 70% of MNPs were cleared from the brain within 7 days. The deposition of MNPs in large animals (by changing the FUS to be low-frequency and planar) demonstrated clearance of MNPs over a similar period. This study established a basis for the feasibility of using FUS to enhance MNPs in the brain and for modifying current commercially and clinically approved MNPs to be used as therapeutics with great potential for treatment of brain gliomas.

**Therapeutic MNPs for FUS-Enhanced Delivery**

Therapeutic MNPs have drawn considerable attention for their potential effect on CNS disease due to their superparamagnetic characteristics that can be guided by an external magnet and simultaneously provide contrast in MR imaging. In the previous section we described the development of MNP delivery into brain with the aid of FUS BBB disruption. Besides concurrent monitoring of the distributions of MNP by T2*-weighted MR imaging, R2 relaxometry MR imaging can be used to quantify the in vivo MNP concentration delivered into the animal brain.

The application of this method was first demonstrated in the normal animal brain to show a significant increase in therapeutic MNP deposition (Fig. 2A). When applying FUS alone, the MNP concentration could be enhanced by up to 50% compared with the contralateral brain. However, with high-magnetization MNPs and the application of external magnetic targeting, the MNPs followed a time-dependent deposition in the sonicated brain, with up to a 20-fold increase compared with the contralateral brain. The calibrated epirubicin concentration in the sonicated/magnetic targeting site reached an upper limit of 21,738 ± 3477 µg/g, whereas FUS alone could only result in 1336 ± 1182 ng/g epirubicin deposition (Fig. 4). Hua et al. similarly demonstrated the success of using magnetic targeting to deliver MNP-BCNU (Fig. 2A) into the brain tumor implant animal cells to confirm the effectiveness of the designed highly magnetized MNP. Tumors shrank markedly after 7 days of treatment with 5 mg/kg of MNP-BCNU with 24 hours of magnetic targeting. In contrast, tumor growth was not inhibited after 7 days by 13.5 mg/kg free-BCNU or 1.68 mg/kg MNP-BCNU with magnetic targeting.

This enhancement in therapeutic MNP delivery was also observed in tumor-bearing animals. Untreated animals showed no MNP accumulation after MNP-epirubicin administration. However, 11,982 ± 2105 ng of MNP-epirubicin was delivered when it was administered in combination with FUS/magnetic targeting, providing a 15-fold higher concentration than the therapeutic range in breast cancer (819 ± 482 ng/g tumor) previously reported for doxorubicin to reach a clinical response rate of 39%.

Control of tumor progression and survival were investigated next (Fig. 5). With a 7-day observation interval, the tumor volume in the FUS/magnetic targeting groups only increased by 106% ± 24% in treated animals com-
pared with a 313% increase (± 103%) in controls, indicating that the combination of therapeutic MNPs with FUS/magnetic targeting provided the most effective means of controlling tumor progression. Moreover, in animal survival, the control and the FUS-enhancement-only treatment resulted in similar median animal survival times (23 and 20 days, respectively), whereas the median survival times were significantly improved by 66% in animals receiving MNP-epirubicin in conjunction with FUS/magnetic targeting treatment (30.5 vs 18.3 days, respectively).

Focused ultrasound combined with magnetic targeting to both passively and actively deliver MNPs thus represents a powerful technique to enhance the delivery of a wide range of macromolecule therapeutic substances into the CNS under the guidance and in vivo monitoring of drug quantification/distribution by MR imaging. Also, the synergistic drug delivery approach provided an improvement of approximately 3.4-fold in the drug’s half-life (from 18 to 62 hours). Because of the longer circulation time of EPEG-MNP-BCNU, its accumulation was excellent (177.33 ± 23.13 μg of Fe ion) and approximately 1.65-fold higher than that of MNP-BCNU (107.72 ± 29.72 μg of Fe ion) after 24 hours of magnetic targeting. This observation supports the idea that EPEG-MNP-BCNU is more suitable than MNP-BCNU for in vivo antitumor studies. The survival rate in animals that received a low dose of BCNU (4.5 mg BCNU/kg in the form of EPEG-MNP-BCNU) was 63 days compared with 50 days in ani-

![Fig. 3. Typical images (upper, coronal plane; lower, transverse plane) showing an animal undergoing FUS to induce BBB disruption, and monitoring the delivery of MNPs. A: Contrast-enhanced T1-weighted imaging after intravenously administered Gd-diethylenetriamine pentaacetic acid contrast agent. B: T2*-weighted imaging before administering MNP. C: T2*-weighted imaging after administering MNP and a 6-hour magnetic targeting procedure. D: R2 map to show the quantitative MNP distribution in the brain. Scale units are sec−1.](image)

![Fig. 4. Typical images for T2-weighted MR imaging (left) and R2 maps (right) of the glioma-bearing animals that did not (A) and did (B) undergo the FUS/magnetic targeting procedure. The FUS/magnetic targeting procedure demonstrates the enhancement of therapeutic MNP retention for at least 6 hours to improve the therapeutic efficacy of glioma treatment. Gliomas are outlined in white.](image)
mals that received a high dose of free BCNU (13.5 mg BCNU/kg). This improvement could greatly enhance the potential of magnetic targeting therapy in clinical applications of cancer treatments.

Besides the above-mentioned highly magnetized MNP design to facilitate local drug delivery into the brain tumor, other attempts have been conducted to further improve the drug activity in the circulation. For example, injected MNPs act as foreign molecules to the body and are still limited by their insufficient stability in aqueous media and marked reticuloendothelial uptake in vivo. In particular, the half-lives of MNPs are fairly short (in the range of minutes) due to their rapid capture and subsequent plasma clearance by macrophages of the reticuloendothelial system, especially in the liver. However, self-protecting EPEG-MNP-BCNU (Fig. 2A) is known to not only have a high capacity for BCNU drug loading and outstanding thermal stability, but long circulation times in vivo as well. The nanosize and excellent dispersion of EPEG-MNP-BCNU allow easier penetration of tissues and more efficient uptake by tumor cells with an enhanced permeability and retention effect.

Dose-Dependent Therapeutic MNPs: In Vivo Considerations

The dose dependency of therapeutic MNPs (Fig. 2A) for treatment of gliomas has also been investigated to determine the optimal enhancement conditions. Normal/glioma rats were subjected to various treatments to compare their efficacy in delivering localized concentrations of nanoparticles to a specific region of the brain. Quantitative inductively coupled plasma optical emission spectrometry analysis of Fe content revealed that FUS alone or magnetic targeting alone only increased therapeutic MNP concentrations by 2-fold relative to the untreated brain. In contrast, the combination of magnetic targeting and FUS resulted in a 10-fold increase in MNP accumulation in the treated region relative to the untreated region.

During a 7-day MR imaging follow-up to track tumor progression, the control, BCNU-only, MNP-only,
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and sham procedure (FUS/magnetic targeting but without MNP delivery) groups showed no tumor shrinkage (Fig. 6). However, for groups receiving high-dose therapeutic MNPs (equivalent to 5 mg/kg of BCNU), tumor volumes were suppressed in the 1st week, both with and without applying FUS/magnetic targeting (~52% and ~97%, respectively). The medium dose (equivalent to 1 mg/kg BCNU) appeared to represent the critical dose for suppression of tumor growth when FUS/magnetic targeting is applied (~79%), but tumors progressed in the sham group (296%). A low dose of therapeutic MNPs did not show repres- sion of tumor progression in either group (163% and 31%, respectively), although the magnetic targeting/FUS group had a relatively slow tumor progression rate. In evaluating animal survival, the median survival time also improved in animals receiving high and medium doses of BCNU-MNPs in conjunction with magnetic/ultrasound treatment; both reached 60 days or above, which was significantly longer than the other experimental groups (all < 33 days).

In the same study, CD68-positive cells were present in the tumor and constituted up to 30% of the tumor mass. The percentage of CD68-positive cells was unchanged in the control and MNP-delivered groups but increased in the MNP combined with FUS/magnetic targeting treatment groups; however, the presence of macrophages increased after sacrificing the treated animals. These findings suggested that MNPs alone do not cause severe immune activity, but the cytotoxic effect of the therapeutic MNPs causes tumor cell death and induces increased macrophage infiltration for the clearance of the necrotic tumor debris as well as the Fe particles.

When summarizing the dosing comparison (Fig. 6), treatment with an effective BCNU dose of 5 mg/kg even without FUS/magnetic targeting was more effective at shrinking tumors than treatment with unbound BCNU at a dose of 13.5 mg/kg, showing an enhanced permeability and retention effect for immobilized chemotherapy drugs on MNPs. The increased drug concentration in tumor cells may result not only from diffusion but also from phagocytosis of drug-bound particles by the cells. Overall, the magnetic/FUS system enhances drug delivery to tumors approximately 5-fold compared with BCNU alone, although the inductively coupled plasma optical emission spectrometry–measured drug amount can have up to a 26-fold enhancement in normal rat brains.

![Fig. 6. A: Ratios of average tumor volume changes in the 1st week after therapeutic MNP treatment under different conditions. BCNU only = 13.5 mg/kg BCNU; high dose = 5 mg/kg therapeutic MNP; medium dose = 1 mg/kg therapeutic MNP; low dose = 0.5 mg/kg therapeutic MNP. B: Representative T2-weighted MR imaging images of the longitudinal glioma follow-up. C: Kaplan-Meier survival curves. Survival improvement at high and medium doses is statistically significant. Redrawn and reprinted by permission of Oxford University Press on behalf of the Society for Neuro-Oncology, from Chen et al.: Neuro Oncol 12:1050–1060, 2010.](image-url)
Conclusions and Perspective

When FUS is used to locally enhance BBB disruption and delivery of therapeutic MNPs, it can be integrated with a novel magnetic targeting approach so that drug delivery proceeds not only through passive but also through active diffusion. The magnet is placed externally to provide magnetic targeting after the FUS exposure and does not contribute any additional risk to the procedure, while significantly enhancing the active attraction of the therapeutic MNP by at least an order in concentration. This innovation raises the possibility of improving therapeutic efficacy of chemotherapeutic agents or reducing the total dose to reduce the circulation toxicity in the body. In addition, the intrinsic nature of MNPs render them sensitive to detection by MR imaging so that the distribution of therapeutic MNPs can be monitored or even quantified during image-guided drug delivery for brain tumor treatment.

In conclusion, preclinical research has shown that FUS is a noninvasive method to enhance the targeted delivery of chemotherapeutic agents through the BBB and into brain tumors. This method allows the chemotherapeutic drug dosage to be increased specifically in the tumor regions, thus significantly suppressing tumor growth and prolonging animal survival. BCNU is already a US FDA-approved chemotherapeutic drug for glioma treatment, and doxorubicin has been approved for ovarian cancers. The successful use of these 2 drugs in FUS MNP preclinical experiments suggests that the procedure could be highly clinically relevant. In addition, chemotherapeutic drugs are delivered intravenously rather than intraarterially or by direct cranial injection/implantation, which makes the treatment more practical in a clinical setting. The procedure is noninvasive, reversible, and can be targeted with greater precision to a specific region of interest in the deep brain under the guidance of MR imaging.

Disclosure

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