Brain Mini-Workshop: Blood Brain Barrier and Targeted Drug Delivery

WORKSHOP SUMMARY

INTRODUCTION

Drugs are currently being developed for the treatment of serious brain disorders including glioblastoma, Parkinson's disease, and Alzheimer's disease. These include chemotherapeutic drugs and those based on neurotrophic factors such as glial cell-derived neurotrophic factor (GDNF), brain-derived neurotrophic factor (BDNF), and neurturin (NTN). Neurotrophic factors have therapeutic potential for neurological disorders such as Parkinson's disease (PD) and Alzheimer's disease (AD), and administration of GDNF and NTN has shown promise in preclinical studies. Despite their safety and effectiveness in preclinical studies, success of chemotherapeutic drugs and neurotrophic factors in clinical trials has been limited in part by the blood-brain barrier (BBB), which naturally protects the brain from potentially harmful agents in the blood. Success of these drugs is also limited by the method of administration. As shown in studies in mice, when NTN is administered systemically, only 0.05% of it reaches the brain. Clinically significant benefits often require direct injection of the compounds into the brain, which is risky, not practical for many patients, and therefor difficult to justify in the early stage of disease progression when the drugs are potentially the most effective. Several researchers are developing drug carriers or new routes of administration, but moving drugs across the BBB remains a challenge.

Focused ultrasound (FUS) is a non-invasive medical technology platform with the potential to serve as an alternative or adjunct for surgery, radiation, or drug delivery for treatment of several clinical indications, including neurological disorders. The ExAblate magnetic resonance imaging-guided FUS (MRgFUS) system (InSightec, Haifa, Israel) has been approved in Europe for the treatment of essential tremor, tremors associated with Parkinson's disease, and neuropathic pain. With this procedure, magnetic resonance imaging is used to guide multiple beams of focused ultrasound energy with extreme precision to heat and destroy a target deep in the brain, in order to alleviate the disease symptoms. Results of a pilot study on FUS for treatment of essential tremor were recently published in the *New England Journal of Medicine*, and a pivotal study is under way at eight sites worldwide to obtain approval by the U.S. Food and Drug Administration (FDA). Clinical trials are also underway for the treatment of PD tremor, PD dyskinesia, and brain tumors. All clinical trials to date for FUS treatment of neurological disorders rely on thermal ablation as the desired biological effect in the tissue. However, FUS has the potential to produce many other biological effects in brain tissue, including the transient disruption of the BBB to enhance drug delivery without damaging the brain when used at much lower energy levels.

The potential of FUS to enhance and target drug delivery to the brain is much less advanced in its development as compared to thermal ablation. Yet several studies are exploring FUS-mediated BBB disruption, and preclinical studies demonstrate that FUS enhances delivery of drugs, proteins and viral vectors across the barrier. However, fewer resources have been devoted to this field. Commercially available systems are optimized for ablation. The optimizations of FUS parameters for BBB disruption, method of drug/molecule administration, and the design of carrier vehicles to facilitate diffusion within the brain, are all important for developing robust clinical CNS therapies. In addition, real-time safety monitoring is available for ablative applications, but no such monitoring has been established yet for BBB disruption and drug delivery.

On September 12–13, 2013, the Focused Ultrasound Foundation, in partnership with the Kinetics Foundation, held a workshop to examine ways to promote the use of FUS-mediated BBB disruption, either alone or with other drug-delivery approaches (eg. viral vectors, nanoparticles), for targeted drug delivery to the brain for treatment of Parkinson's disease, glioblastoma, and Alzheimer's disease. Following research presentations and a discussion on the clinical perspective, workshop participants developed potential roadmaps for moving the field forward. The workshop also was intended to foster collaboration by bringing together a multidisciplinary group of luminaries from fields including focused ultrasound, microbubble and nanoparticle development, drug/gene/protein delivery methodology, and neurosurgery.

RECENT WORK

FUS for BBB Disruption

Several groups are investigating the use of FUS in combination with microbubbles to transiently open the BBB in preclinical models including mouse, rat, rabbit and non-human primate. Different FUS systems and a range of FUS parameters are being used at the various sites as summarized briefly here.

Kullervo Hynynen's group at Sunnybrook Health Sciences Centre (Toronto, ON, Canada) has designed an MRgFUS system with a focused transducer that can quickly scan multiple locations (2-minute sonication, 10 ms bursts, PRF 2 Hz, simultaneous infusion of Definity microbubble [0.02 ml/kg] at 1 minute), using the ultraharmonic as a controller and internal calibration of burst pressure.[1] They also have developed a two-photon microscopy technique to detect BBB disruption and assess individual vessels during disruption by correlating dye leakage kinetics with acoustic pressure and vessel size.[2] Hynynen and colleagues have found that the BBB can be opened with no apparent tissue damage at a range of 0.18 to 0.6 MPa. They also have applied the MRgFUS system and two-photon microscopy technique to a mouse AD model and found that affected vessels in the brain show altered kinetics and that plaque size is reduced following FUS. FUS work has also been performed in rabbit and rat models.

[1] O'Reilly and Hynynen Radiology 2012;263:96-106.
[2] Nhan et al. J Controlled Release 2013 Sep 2 Epub ahead of print.

Nathan McDannold's group at Brigham and Women's Hospital (Boston, MA, USA) has conducted FUS-mediated BBB disruption in monkeys,[3] using the InSightec ExAblate 4000 low-frequency (220 kHz) system, with a 1024-element phased array transducer and a 30 cm hemisphere (50 s sonication, 10 ms bursts, acoustic power 0.5 to 10W [1W = 0.22 MPa], total treatment duration of 5 minutes) and infusion of microbubbles. Beam steering allows them to include nine spots per sonication, with a new location every 120 to 400 ms and a PRF of 0.25 to 1.1 Hz at each location. McDannold and colleagues have sonicated 185 targets across seven monkeys and observed BBB disruption with no tissue damage at 1W. The size of disruption varies across locations, depending on the pressure amplitude, and BBB disruption in white matter is evident not by MR contrast, but by dye leakage. McDannold's laboratory has found a predictive relationship between the strength of the harmonic and the magnitude of MRI contrast enhancement,[4] and it has developed a method of feedback monitoring based on passive cavitation mapping.[5]

Both Hynynen and McDannold have conducted work using FUS-mediated BBB disruption to enhance delivery of molecules to the brain. Hynynen's group has demonstrated the ability of FUS-mediated BBB disruption to facilitate delivery of stem cells or viral vectors, and their work in the mouse AD model has shown improvements in behavior following MRgFUS combined with administration of microbubbles and an anti-Aβ antibody.[6] McDannold and colleagues have conducted three weekly FUS treatments (10 ms bursts, low-duty cycle for 40 to 60 seconds, peak negative pressure amplitude of 100 to 1000 kPa, ultrasound contrast agent applied at one- to two-times the clinical dose) with liposomal doxorubicin in a rat glioma model. Seven of eight treated rats showed a 100% improvement in survival following treatment. In another rat model in which breast cancer cells had been introduced into the brain, six weekly treatments of FUS and trastuzumab administration improved survival for 50% of the animals, as compared to those animals treated by FUS or trastuzumab alone. Additionally, McDannold's group has used dynamic contrast-enhanced MRI to observe a half-life of approximately 2 hours for BBB disruption. They also have found that repeat BBB disruption can be performed safely about an hour later.

Elisa Konofagou's group at Columbia University (New York, NY, USA) has shown that BBB disruption at 0.8 MPa facilitates diffusion of gadolinium across the BBB to the hippocampus,[7] that the degree of BBB disruption can be controlled through the size of microbubbles used,[8] and that the BBB can be disrupted with very short pulses in as few as three cycles. Recent work has focused on FUS-mediated BBB disruption and administration of Definity microbubbles (1.5 MHz frequency, 10,000 to 30,000 cycles, PRF of 10 Hz, total sonication duration 60 seconds, at a pressure of 0.3 to 0.6 MPa) to facilitate delivery of systemically administered NTN, GNDF, or BDNF in mouse PD or AD models.[9] Delivery of BDNF to hippocampus is enhanced in the sonicated region, as suggested by activation of downstream targets. Similarly, delivery of NTN to the substantia nigra and caudate putamen is enhanced, and NTN also appears to enter the

[3] McDannold et al. Cancer Res 2012;72:3652-63.
[4] Arvanitis et al. PLoS One 2012;7:e45783.
[5] Arvanitis et al. Phys Med Biol 2013;58:4749-61.
[6] Jordao Expt Neurol 2013;248:16-29; Jordao et al. PLoS One 2010;5:e10549.
[7] Choi et al. Phys Med Biol 2007;52:5509-30; Choi et al. Ultrason Imaging 2008;30:189-200.
[8] Choi et al. J Cereb Blood Flow Metab 2011;31:725-37.
[9] Baseri et al. Phys Med Biol 2012;57:N65-81.

neurons. However, experiments with GDNF have not been successful. Although BBB disruption is apparent by MRI, GDNF is not delivered, for reasons that are unclear. Konofagou's group is now exploring BBB disruption to enhance targeted drug delivery through the skull to the hippocampus in monkeys.

Carrier Vehicles for Drug Delivery

Even with effective BBB disruption via FUS plus microbubbles, it may be difficult to deliver a clinical "dose" of the therapeutic agent to the desired region of the brain. Therefore, several groups are investigating the "packaging" of drugs, proteins, or genes using nanoparticles, viral vectors or microbubbles, to enable more efficient delivery to and throughout the desired region of the brain.

Nanoparticles. The primary challenges for nanoparticle design involve penetration into the brain parenchyma and potential trapping of particles because of adhesion.[10] To circumvent these challenges, Justin Hanes' group at John Hopkins University (Baltimore, MD, USA) which has designed several nanocarriers for drug delivery to a variety of organs, has developed densely pegylated, minimally invasive nanocarriers.[11] Using these nanocarriers, they have found that the pore size in mucus is larger than previously estimated, about 0.5 microns,[12] and that unexpectedly large nanoparticles, larger than 100 nm, that are densely coated with PEG can diffuse rapidly through the brain.[13] These nanoparticles are fairly stable and can be stored at room temperature for an extended amount of time. Recent work has focused on the use of pegylated nanoparticles in a glioma model.

Work in Rich Price's laboratory at the University of Virginia (Charlottesville, VA, USA) has focused on ultrasound-targeted delivery of drug- and gene-bearing nanoparticles. His group has demonstrated that, in skeletal muscle, enhanced delivery following ultrasound (1 MHz transducer, 1.2 MPa peak negative pressure, inertial cavitation likely) is significantly higher with a microbubble-nanoparticle composite agent than with co-injection of microbubble and nanoparticle. In a mouse subcutaneous glioma tumor model, the group has demonstrated that treatment with ultrasound and a composite agent bearing 5-fluorouracil slows tumor growth and improves survival. However, histology also reveals clumps of nanoparticles, suggesting that this strategy might not be suitable for delivery across the BBB.

Hanes and Price have collaborated to explore the use of MRgFUS and microbubbles to deliver brain-penetrating nanoparticles. At 1 hour following sonication (1.14 MHz, peak negative pressure 0.4 to 0.6 MPa, pulse interval 2 s, pulse duration 10 ms, total treatment time 2 min, microbubble bolus dose 10^5 bubbles/g), the amount of nanoparticles delivered and the percentage of vessels opened are significantly enhanced at 0.6 MPa. MR intensity correlates with the

[10] Barchet and Amiji. Exp Opin Drug Deliv 2009;6:211-25; Omidi and Bara. BioImpacts 2012;2:5-22
[11] Lai et al. Proc Natl Acad Sci USA 104:1482-7; Wang et al. Angew Chem Int Ed Engl 2008;47:9726-9; Tang et al. Proc Natl Acad Sci USA 2009;106:19268-73; Lai et al. Proc Natl Acad Sci USA 2010;107:598-603; Yan et al. Angew Chem Int Ed Engl 2011;50:2597-600; Ensign et al. Adv Mater 2012;24:3887-94; Nance et al. Sci Transl Med 2012;4:149ra119; Ensign et al. Sci Transl Med 2012;4:138ra79; Kim et al. Angew Chem Int Ed Engl 2013;52:3985-8.
[12] Lai et al. Proc Natl Acad Sci USA 2010;107:598-603.
[13] Nance et al. Sci Transl 2012;4:149ra119.

amount of nanoparticle, but this will need to be refined for more quantitative MR estimates of nanoparticle delivery. In another study, Hanes and Price have demonstrated that in skeletal muscle, ultrasound (1 MHz, peak negative pressure 0.6 MPa, pulse duration 0.1 ms, pulse frequency 5 s) combined with co-injection of microbubble and a PEI-PEG-DNA nanoparticle construct results in enhanced transfection 7 days following treatment. Transfection is better with smaller microbubbles, but under these parameters, these microbubbles likely undergo inertial cavitation. Secondary uptake of carrier by the cells has been observed, but whether this will happen in the brain or at lower pressures is not clear. The potential toxicity associated with PEI has not been addressed. Work has not yet been completed with ultrasound and drug-loaded nanoparticles.

Microbubbles. Microbubbles range from 1 to 10 microns in diameter and oscillate in the presence of ultrasound waves. They separate the mechanical effects of FUS from the thermal effects and are necessary for BBB disruption. Mark Borden's laboratory at UC Boulder (Boulder, CO, USA), which focuses on microbubble design, has size-selected microbubbles via centrifugation[14] and manipulated the rigidity and stability of bubbles by changing diacyl chain length in the diacyl phosphatidylcholine coat.[15] The laboratory has also developed polyplex bubbles,[16] in which polyethylenimine is pegylated, thiolated, and attached to malemide groups on lipid-coated bubbles, and demonstrated in mouse models that these bubbles improve gene delivery. Borden and his colleagues also have developed gadolinium-coated bubbles[17] and found that MR can be used to monitor bubble fragmentation and cavitation. Other types of microbubbles include gold-coated microbubbles,[18] which can be visualized with dual-modality imaging and potentially guide agent delivery to the brain, and cloaked microbubbles,[19] which employ lipid-anchored polymer chains of different lengths to shield ligand from blood complement and reveal it when it reaches its target. Lung surfactant bubbles also have been developed to enhance drug load,[20] but these are not optimal for hydrophilic drugs.

The work in Sasha Klibanov's laboratory at the University of Virginia focuses on increasing the therapeutic index of drug-conjugated microbubbles. He and his colleagues have designed a liposome-carrying microbubble and demonstrated *in vitro* that it breaks and releases its contents with ultrasound. Similar results have been observed *in vivo*, in a collaboration with Philips Medical, testing ultrasound (spiral pattern, repeated every 6 minutes at 1.2 MHz, 2 MPa, 10,000 cycles, 10 Hz pulse repetition frequency [PRF]) combined with a fluorescent doxorubicin-conjugate liposome microbubble (injected dose 3 mg/kg) in a mouse model harboring a subcutaneous colon adenocarcinoma in the hind leg. In addition, the tumor growth rate in this model is reduced transiently following treatment. More recently, to circumvent the potential effects of microbubble destruction on blood flow and drug delivery, Klibanov and colleagues have combined nondestructive ultrasound (1 MHz, continuous insonation, 0.4 W/cm^2, 10 min) with selective microbubble-drug targeting, using a biotinylated microbubble conjugated with doxorubicin-carring liposomes. A high drug dose (infused dose of 6 mg/kg) was delivered with

[14] Feshitan et al. J Colloaid Interface Sci 2009;329:316-24.
[15] Garg et al. Biomaterials 2013;34:6862-70.
[16] Sirsi et al. J Control Release 2012;157:224-34.
[17] Feshitan et al. Biomaterials 2012;33:247-55.
[18] Dove et al. Soft Matter 2013.
[19] Borden et al. Mol Imaging 2013;12:357-63.
[20] Sirsi et al. Theranostics 2013;3:409-19.

no observable toxicity, and statistically significant inhibition of tumor growth. Targeted transfection was achieved by combining antibody-carrying microbubble, plasmid and unfocused ultrasound, in a model of Crohn's disease. Transfection was observed in the targeted area[21]. Other ongoing work involves the use of catheters to inject drug while generating bubbles at the injection site from air or perfluorocarbons, with the goal of using ultrasound to release drug locally.

Artenga, Inc., and the National Research Council of Canada (NRC) are planning a study to combine clinically scalable microbubbles with MRgFUS to deliver NTN and other factors across the BBB. The therapeutic microbubble developed by Artenga has been used in studies of FUS-mediated BBB disruption and in the delivery of targeted chemotherapy to prostate tumors in a mouse model.[22] NRC has conjugated proteins to microbubbles and used imaging as a surrogate marker for delivery across the BBB of protein-targeted, liposome-encapsulated doxorubicin.[23] The planned study will target the substantia nigra and putamen, with specific aims to optimize drug loading and FUS-mediated disruption, confirm bioactivity of the microbubble, quantify delivery and biodistribution for a range of doses, confirm *in vivo* receptor activation, assess preclinical response in a PD model, and assess safety and toxicology. At the suggestion of workshop participants, the caudate may be considered.

Other Drug Delivery Methodologies

Adeno-associated virus (AAV) vectors. Human AAV, a widespread, non-pathogenic, single-stranded DNA virus, can be useful in gene therapy. Recombinant AAV vectors enable anatomical specificity, and long-term, stable and controlled transduction in tissue, including brain. The AAV2 serotype has been studied in several clinical trials for brain disorders. Although this serotype can target smaller areas, its transduction efficiency is low. Using a green fluorescent construct in a rat model, Corinna Burger's group at the University of Wisconsin (Madison, WI, USA) has found that AAV1 and AAV5 transduce with higher efficiency and distribute more widely throughout the entire brain.[24] Among other serotypes that have been characterized,[25] AAV9 can cross the BBB with the aid of FUS, but it shows less specificity.

In collaboration with Konofagou, Burger's group is using FUS and AAV1 or AAV2 to target the striatum. Again, they have found wide distribution, even to areas outside the targeted region. They also have demonstrated that MR contrast agents such as gadoteridol increases distribution of AAV1 in the hippocampus[26] and AAV5 in the striatum.[27] The mechanism of enhanced distribution is not clear.

Several clinical trials of AAV-based gene therapy are under way. However, direct gene therapy into the brain requires an invasive procedure (needle positioned into the parenchyma of the brain) in which current drug infusion technologies do not prevent backflow or transgene

[21] Tlaxca et al. J Control Release 2013;165(3):216-25.
[22] Goetz et al. PLoS One 2012;7:e52307.
[23] Iqbal et al. Methods Mol Biol 2011;686:465-81.
[24] Burger et al. Mol Ther 2004;10:302-17.
[25] Cearley and Wolfe. Mol Ther 2006;13:528-37.
[26] Hullinger et al. Gene Ther 2013; 20:1172-7
[27] Osting et al. Mol Ther Methods Clin Dev 2014; in press.

expression in unwanted regions along the needle track. In addition, there is no way to inactivate the transgene if adverse effects occur. Because of these concerns, the FDA does not allow for clinical trials at earlier stages of neurological disorders, before massive loss of neuronal projections from the substantia nigra to the striatum are lost.

Intranasal delivery. Intranasal delivery of agents bypasses the BBB entirely by taking advantage of transport along olfactory and trigeminal nerves from the nasal cavity into the brain. Barbara Waszczak's laboratory at Northeastern University (Boston, MA, USA) studies intranasal delivery of GDNF and GDNF plasmid nanoparticles in a rat PD model. Although intranasally administered GDNF increases GDNF levels in the brain and appears to be neuroprotective in the rat PD model, only a small amount of the administered dose, about 0.001% reaches the brain. Waszczak's group has also collaborated with Copernicus Therapeutics, Inc. on a study of intranasal administration of a pegylated, poly-lysine plasmid-GDNF nanoparticle. Nasal penetration of these nanoparticles causes transfection and expression of the protein throughout the brain one week after administration, and also appears to be neuroprotective in the rat PD model. Protein expression occurs largely in cells adjacent to the vasculature endothelium, consistent with other findings that intranasally administered agents distribute through perivascular flow.[28] It appears that GDNF plasmid nanoparticles, like other agents administered intranasally, are taken up preferentially by the pericytes lining the capillaries. Studies are needed to determine whether FUS can 1) improve overall intranasal delivery to the brain, for example by increasing uptake from the nasal cavity, and/or 2) increase tissue penetration of the nanoparticles, by promoting escape from the perivascular space, thereby promoting GDNF production at neural targets in brain.

TRANSLATION TO THE CLINIC

The eventual clinical adoption of FUS for BBB disruption and enhanced drug/gene delivery for the treatment of CNS disorders will require the approval of several "parts" of this combination therapy. These include the FUS system (medical device), drug and/or carrier vehicle, and the microbubbles necessary for safely opening the BBB. An assessment of the current status of each of these areas, as briefly presented below, helps to identify the gaps that must be addressed with future work.

Current Status

Several clinical studies are exploring drug delivery to the brain in neurological diseases. Ceregene has conducted a trial of AAV2-NTN (CERE-120) for PD. UCSF is expecting to soon begin a trial of AAV2/AADC (aromatic L-amino acid decarboxylase). Medgenesis is conducting a pilot study of GDNF protein infusion in six patients, and a larger study with an additional 36 patients is expected to begin in fall 2013. As pointed out by Waszczak, clinical studies also are exploring intranasal delivery of proteins such as insulin to address CNS effects. Studies of trophic factors in PD have been disappointing so far, most likely because they must wait until too late a stage in these diseases, when the neuronal projections from the substantia nigra to the striatum are lost.

[28] Dore-Duffy et al. Curr Pharm Des 2008;14:1581-93; Krueger and Bechmann. Glia 2010;58:1-10.

Clinical studies are ongoing for FUS ablation of targets deep in the central region of the brain (thalamus, pallidum) for treatment of essential tremor and PD. All studies so far have used the ExAblate 650 kHz system. The Focused Ultrasound Foundation and the University of Virginia are collaborating to determine the preclinical feasibility of the ExAblate 650 kHz and 220kHz systems to treat targets, including the hippocampus, that reside away from the center of the brain. Results from this study are expected soon.

The Definity microbubble has been approved for clinical use in diagnostic assessments using ultrasound imaging.

The Clinical Perspective: What Is Needed

As pointed out by Graeme Woodworth, one critical question for any treatment study focused on the brain involves the effects of that treatment on brain function. Studies presented during this workshop have demonstrated that FUS-mediated BBB disruption exerts little to no damage or effects on high-level brain function. As more steps are taken to move FUS-mediated BBB disruption and drug delivery to the clinic, however, investigators will have to consider which disease to begin with, what is needed for trial approval, and whether the technology is ready for the clinic. For selecting which disease will serve as a starting point, Woodworth suggests considering whether the disease is of relatively high prevalence, what the existing treatments are, whether it is focal or diffuse, and whether its potential targets are deep or superficial. Lessons can be learned from development of ablative FUS for essential tremor.

With respect to determining what is needed for trial approval and assessing whether the technology is ready, Woodworth suggests that many studies, while useful, have focused on numerous variables at once. Attempting to explore the feasibility and safety of FUS-mediated BBB disruption at the same time as assessing which drugs are beneficial, may be problematic. Even when focused primarily on FUS-mediated BBB disruption, studies are aiming to determine the accuracy of disruption, particularly for superficial targets near the skull; identify ways to monitor safety and tissue effects; and establish safety and efficacy. Trials that focus on one specific variable at a time might speed translation of FUS-mediated BBB disruption and ultimately, FUS-enhanced drug delivery to the clinic.

Close to the Clinic

As discussed by Ryan Alkins, the University of Toronto and Sunnybrook Health Sciences Centre have received conditional approval to conduct a prospective, single-arm clinical trial exploring the safety and feasibility of transcranial MRgFUS-mediated BBB disruption and intravenous administration of Definity microbubble in doxorubicin treatment for brain tumor. Ten patients who have not received previous radiation will receive infusions of liposome-doxorubicin on the day they are scheduled for surgery, followed by CT targeting and FUS-mediated BBB disruption using the InSightec ExAblate Neuro system (3 to 5W acoustic power, 0.74% duty cycle, 50 s total duration, with electronic steering of the beam through a nine-point grid with 3 mm spacing). Patients will undergo surgery later that day, within hours after FUS. FUS-mediated BBB will be used on only a small portion of the tumor, less than 2.5 cm in size, and a small area adjacent to the tumor. Hynynen is a collaborator on this study, which will hopefully demonstrate: (1) that

FUS can safely open the BBB, (2) that the 220kHz ExAblate system is safe, and (3) the pharmacokinetics and drug concentration in the tumor.

A potential follow-on trial at Sunnybrook would be similar except more areas of the brain (both in the tumor and adjacent) would be sonicated, and the treated tissue would be left in place for some time after FUS treatment and before resection. This study could provide further safety data including the time (after sonication) to BBB closure, as well as preliminary efficacy data for FUS enhanced delivery of drug to the tumor and its therapeutic effects.

It should be noted that FUS-mediated BBB disruption and drug delivery is unlikely to replace surgery completely for brain tumors, as surgery will still be needed for diagnosis and debulking. However, Alkins envisions a treatment approach in which diagnostic and debulking surgery is followed by FUS-mediated BBB disruption, then radiation or chemotherapy. Studies are needed to determine whether post-operative BBB disruption is safe and how the irradiated vasculature could affect FUS parameters for BBB disruption.

Woodworth has proposed a study exploring the use of FUS-mediated BBB disruption to mark brain areas for surgical resection in patients with brain tumors. During tumor resection, surgeons typically remove areas around the tumor as well, because these areas have been infiltrated. Work from Price's laboratory has demonstrated the ability of FUS to disrupt the BBB in infiltrated areas, and the proposed study would use approved dyes, such as methylene blue, to better visualize these areas. Such a study could enroll large numbers of patients and focus specifically on optimizing the accuracy, monitoring, and safety of FUS in humans. Thus it could speed translation of FUS-mediated BBB disruption to the clinic, and it could serve as a starting point for other studies investigating drug delivery. Workshop participants suggest that this study could be complementary to the abovementioned Sunnybrook study.

The planned trials in Canada hint at another approach: using incremental studies in other countries to smooth the path toward approval in the United States. A study in Zurich exploring FUS ablation therapy for neuropathic pain paved the way for pilot studies and eventual approval (likely 2015) in the United States. Lessons could also be learned from Ceregene, who were able to move their trials forward by exploring the introduction of AAV and drug payloads in a logical, stepwise manner.

On the Horizon

FUS-mediated BBB enhancement and drug delivery. In other areas, the use of FUS-mediated BBB disruption to enhance drug delivery to the brain is still on the horizon, though expected to reach the clinic within two years. Hynynen's work has demonstrated that repeated BBB disruption by FUS with microbubbles can open the BBB, reduce plaques, and rescue behaviors in animal AD models. No drug is needed for these effects to occur. Preclinical studies in large animals are necessary to demonstrate further safety before moving on to a clinical study.

More preclinical work is needed with the ExAblate 220 kHz system to determine whether the base of the skull can be targeted with FUS, the effects of treating near the base, the optimal placement of the helmet, and the thermal envelope of this system. Other preclinical studies are needed to optimize drug administration to enrich delivery to the brain and minimize exposure to

the periphery. In addition, a clinically significant dose of therapeutic agent can be achieved in the brain for tumors, but AD and PD are more challenging. For example, with late-stage AD, lesions are diffuse across the entire brain; thus the percentage of BBB that should be opened must be defined. The brain regions affected at early stages are known, and administration of drug at these stages is ideal. However, early stages are harder to diagnose, and the FDA is less likely to approve BBB disruption at these stages.

As noted by Konofagou, systemic administration of neurotrophic factors is not feasible in larger animals or humans, because a larger dose is needed to overcome the large percentage of drug lost to the periphery, increasing the cost and potentially causing side effects. Workshop participants thus suggest a Phase 0 study of FUS-mediated BBB disruption (using the InSightec 220 system), combined with introduction of a limited dose of CERE-120 through the carotid artery to maximize the amount reaching the brain. AAV2/AADC could also be used as an agent, as there is a PET tracer for it and there may be less concern with periphery-associated adverse events. In addition to minimizing the potential peripheral effects, such a study would employ components that already have been approved. It also could build on data from preclinical studies of CERE-120 or AADC in non-human primates. However, preclinical studies of the efficacy of FUS combined with CERE-120 (or other viral vector) in a PD model, perhaps in rhesus macaque, would also be needed, and these studies would need to include careful attention to the toxicology.

Other potential areas for clinical study of FUS-enhanced drug delivery could include lysosomal storage diseases, for which therapies have been approved, and Huntington's disease. FUS has been used to enhance delivery of silencing RNA (siRNA) across the BBB and block expression of Htt protein expression in "normal" animals; a next step could be to try this in a rat model of Huntington's. Gaucher's disease could also be an option, as the FDA is more open to research in orphan diseases. Preclinical studies, including toxicity in large animals, would be needed before progressing to clinical studies.

Nanoparticles. Another promising route to the clinic, with a likely longer time horizon, involves brain-penetrating nanoparticles loaded with chemotherapeutic agents or genes. A pegylated nanoparticle with cisplatin is already in clinical studies, and other nanoparticle-agent combinations could be added to the University of Toronto/Sunnybrook study. This route offers the advantages of targeting to the brain, enhanced drug stability, and improved distribution. Moreover, equal efficacy could be achieved with 10- to 100-fold less drug, minimizing the risk for adverse effects.

Delivery of transgene-bearing nanoparticles is advantageous for gene delivery, because duration of expression can be controlled, the particle can be changed to adjust distribution, and there are no size limitations on the DNA packaged into the nanoparticle. However, transgene-bearing nanoparticles would require re-administration for continuous transgene expression; at present, transgenes are expressed for up to 6 months. Reporter gene expression data from Hanes and Price's collaboration are expected within months, and Hanes speculates that clinical studies are possible within 3 to 5 years, depending on whether proof-of-concept and toxicology trials are conducted in parallel or sequentially. Financial support will be needed for Good Laboratory Practice (GLP) and GLP toxicology studies. In addition, simplicity is key; loading nanoparticles with more functions will place them farther away from the clinic. Experts in PD and

nanotechnology could collaborate, and perhaps engage with a representative from the FDA, to move this area forward.

Viral vectors. The use of AAV vectors is also advantageous for gene therapy, particularly because it requires only one administration. However, this vehicle is limited by small coding capacity, a limited number of entry points across the BBB, and wide distribution of some serotypes. A preclinical, systematic comparison of AAV serotypes with or without FUS, measuring the amount of virus and transgene expression in the periphery, has been suggested. Burger is also working on ways to re-target capsid to minimize peripheral effects, and one of Hynynen's collaborators is exploring the point at which wide distribution becomes detrimental. In addition, Hynynen is investigating FUS combined with AAV9 in models of spinal muscular atrophy. Some work has been done in conjugating viruses to microbubbles, but the results have been mixed.

Microbubble development for drug delivery. Microbubble-protein conjugates are another potential route to the clinic for the FUS field, though likely at least three to five years in the future. Medgenesis data on GDNF will likely be available in 2015. A preclinical study in China has demonstrated that MRgFUS and microbubbles improve delivery of GDNF across the BBB and included neuronal assays to measure GDNF activity.[29] NRC is working to improve protein packing for the microbubble conjugate used in the Chinese study, and Artenga is planning preclinical studies that will include toxicology and organ data. Preclinical work is also needed to assess the pharmacokinetics and pharmacodynamics of GDNF, to demonstrate that GDNF can be attached to a microbubble, that it remains bioactive when injected, and that it causes no other unintended problems. After completion of this preclinical work, a clinical trial could then be considered.

It should be noted that microbubble-protein conjugates require repeated administration, the small surface of microbubbles requires the use of proteins with small therapeutic doses, and the relationship between the stable/inertial cavitation boundary and lipid microbubble destruction is not clear. Studies are also needed to address issues of compatibility between the bubble shell and protein of interest.

Intranasal delivery. Farther out on the horizon is the use of FUS to enhance intranasal delivery to the brain. The study of intranasally administered insulin is already approved by the FDA, but preclinical work is needed to determine whether FUS can enhance delivery following intranasal administration. Suzanne Craft, who is conducting the studies of intranasal insulin for patients with AD,[30] is a potential partner. Chongqing Haifu has a clinical device that has been approved for allergic rhinitis, and Impel Neuropharma is manufacturing a device for intranasal delivery. In addition, Hanes has offered nanoparticles developed in his laboratory for Waszczak and others to use in studies of FUS and intranasal delivery, and the Copernicus nanoparticles used by Waszczak are already dosed for humans. Representatives from Copernicus could be involved in further discussions.

[29] Wang et al. PLoS One 2012;7:e82925.
[30] Craft et al. Arch Neurol 2011;69:29-38.

Technological Limitations/Needs

Despite the promise of FUS-mediated BBB disruption to enhance drug delivery, translation to the clinic is hampered by several limitations. In the United States, only one FUS system is approved for clinical trials, and it is intended for ablative therapy. When using the system for BBB research, it is limited by the number of spots per sonication and how fast power can be assessed and modulated. The hydrophones embedded in the transducer do not record small increments of time, and the broadband and harmonics are not very clear. Moreover, the need for MRI in planning and follow-up makes the system impractical for repeated treatments.

The amount of power required for BBB disruption is much lower than that used in commercially available systems, and a lower frequency is needed to improve steering capability and minimize skull aberrations. Although the 220 kHz system could address some of these challenges, this system is available at only three sites worldwide. The animal systems described during this workshop appear to be better suited for research in FUS-mediated BBB disruption.

Regulatory approval also presents a hurdle. Unlike Canada, where systems built within hospitals or centers can be used with institutional approval, the United States requires investigational device exemptions (IDEs) for studies using experimental devices. Companies might not seek an IDE for clinical applications they feel will never reach the market, but without company sponsorship, the cost to investigators for the IDE process is prohibitive.

OUTCOMES AND NEXT STEPS

At the conclusion of the workshop, a potential roadmap forward for clinical indications including glioblastoma, Alzheimer's disease and Parkinson's disease had been developed, as presented throughout this document. The future preclinical studies necessary to fulfill this clinical roadmap were also identified. Longer-term preclinical studies necessary to move the field of focused ultrasound plus nanocarriers or conjugated-microbubbles for enhanced drug/gene/protein delivery toward clinical trials were also identified. Additionally, new multi-disciplinary research collaborations were fostered. The Focused Ultrasound Foundation encourages applications for funding collaborative projects in this field. FUSF will also lead efforts to promote further awareness of this field with other potential funding organizations (e.g. disease-specific foundations) and seek co-funding opportunities to support collaborative projects. FUSF also intends to coordinate smaller group meetings, with the inclusion of more clinical experts, to further define roadmaps for specific clinical indications.

ABBREVIATIONS

AADC	aromatic L-amino acid decarboxylase
AAV	adeno-associated virus
AD	Alzheimer's disease
BBB	blood-brain barrier
BDNF	brain-derived neurotrophic factor
FDA	U.S. Food and Drug Administration

FUS	focused ultrasound
GDNF	glial cell-derived neurotrophic factor
GLP	Good Laboratory Practice
MRgFUS	magnetic resonance imaging-guided focused ultrasound
NTN	neurturin
PD	Parkinson’s disease
PRF	pulse repetition frequency

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