Improved outcome of targeted delivery of chemotherapy drugs to the brain using a combined strategy of ultrasound, magnetic targeting and drug-loaded nanoparticles

"...understanding of the exact mechanisms of the observed transport by ultrasound and magnetic targeting, as well as a detailed assessment of tissue damage, are critical in determining the clinical translatability of the technique for delivery of chemotherapeutic drugs to treat brain tumors."

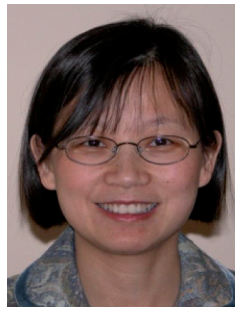
Keywords: blood–brain barrier ■ brain tumor ■ cavitation ■ chemotherapy ■ drug delivery ■ magnetic nanoparticle ■ magnetic targeting ■ microbubble ■ MRI ■ ultrasound

Ultrasound (US) application has been demonstrated to be capable of generating localized opening of the blood–brain barrier (BBB) and enhanced transport of therapeutic agents such as chemotherapeutic drugs and antibodies into the brain in animal models [1–11]. Liu *et al.* recently reported new results of a comprehensive study using a combined approach of US technique, magnetic targeting (MT), and drug-loaded magnetic nanoparticles (MNPs) to improve delivery of epirubincin (a chemotherapeutic drug used to treat malignant tumors) into a brain tumor in a rat model [12]. This is the first study to demonstrate the synergistic benefit of US-mediated BBB opening and additional mechanisms, and it illustrates a promising new venue for future development of US-mediated drug delivery across the BBB to the brain. The synergistic delivery is achieved by using MNPs as a multifunctional agent for conjugating epirubicin, MT and MRI monitoring of delivery. MRI monitoring of US-induced BBB opening and MNP deposition in the brain was performed and corroborated with post-US assay results of MNP accumulation and epirubicin concentration in brain tissue. By comparing the delivery outcome (MNP accumulation and epirubincin concentration) in normal brain and tumor by US alone, MT alone and a combination of US plus MT, the authors clearly showed that the combination of US and MT achieved significantly increased delivery and therapeutic outcome in terms of tumor progression and animal survival.

Drug delivery to the brain across the BBB

Treating malignancies in the brain, including primary brain cancer and brain metastases, which occur in a significant percentage of patients with common malignancies including lung, breast and colon cancer [13], continues to be a critical challenge [13–15]. In particular, pharmacological therapy often exhibits poor outcomes due to the presence of the BBB [16]. The BBB is mainly comprised of brain microvascular endothelial cells with extremely low permeability, high *trans*-endothelial electrical resistance, and low occurrence of pinocytotic vesicles [17–19]. The tight junctions (TJs) between the endothelial cells restrict paracellular passage of water-soluble or hydrophilic substances from the blood to the brain parenchyma. Transcellular transport is limited by an ensemble of enzymes, receptors, transporters and efflux pumps associated with the multidrug-resistance pathways [19]. The BBB ensures that substances in the general circulation do not readily and freely reach the brain parenchyma, but it also blocks many systemically administered drugs from entering the brain, thus they cannot achieve therapeutic concentrations. The impermeability of the BBB remains the most important factor limiting delivery of therapeutic agents to the brain [14,20].

Strategies to deliver drugs to the brain across the BBB commonly target the various pathways of BBB transport. Chemical modification of a drug into a more lipophilic form increases its permeability to the BBB through the transcellular pathway, although the permeability of the



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drug molecules increases over the entire brain, and even other organs throughout the body. Increasing lipid solubility may also result in the compound becoming a substrate for active efflux pathways such as the permeability P-glycoprotein (P-gp). Development of more hydrophobic drug analogs or linkage of an active compound to a compound capable of passage across the BBB, as well as re-engineering of biopharmaceuticals with molecular Trojan horses [16,21] can utilize various endogenous transport mechanisms of the BBB. However, these methods rely on adaptation to each type of drug molecule and specific BBB transporters, thus involving time-consuming and costly processes. Intra-arterial injection of hyperosmotic mannitol or other hyperosmotic solutions has been used to open the TJs [22,23], yet shrinkage of the endothelial cells also causes diffusive BBB disruption in the highly perfused brain. Direct injection procedures such as convection-enhanced delivery [24,25] can deliver therapeutics that bypass the BBB, but the invasive introduction of catheters that traverse untargeted brain tissue can cause unnecessary damage and increase the risk of complication. Despite much effort, the brain-delivery techniques clinically used and many that are currently undergoing research have not yet provided practical or satisfactory solutions to drug-delivery across the BBB.

Recently, focused US application following intravenous administration of preformed microbubbles has been demonstrated to generate localized and transient BBB opening, resulting in delivery of various agents from chemotherapy drugs to antibodies into the brain [1–10,26]. With transcranial application [27–29] and focal deposition of non-ionizing US energy, this technique provides a versatile and compelling strategy for localized BBB opening and targeted drug delivery to the brain in a noninvasive fashion [30].

A combined strategy to achieve improved outcome of brain delivery

Previously, US-mediated delivery of therapeutic agents relied on passive diffusion of the circulating agents into the brain parenchyma through the US-induced BBB opening [1–10,26]. However, transport of therapeutics into the brain can still be limited owing to the low intrinsic diffusion coefficient of the agent, limited circulation concentration of the agent, and an elevated interstitial pressure in the tumor. This study explored a combined strategy to address some of these issues and has achieved improved outcome of epirubucin delivery to tumors in the brain in a rat model.

■ Animal model & US-induced BBB disruption

Brain tumors were induced by injection of cultured C6 tumor cells in rats with a bodyweight of 300–400 g. This study used a similar protocol for US-induced BBB opening as in previous studies [1–10]. Bolus injection of SonoVue® SF6 microbubbles (Braco, diameter of 2–5 μm) was performed before US application. However, this study provided no assessment of the presence and concentration of microbubbles in the brain to reveal direct information of US-induced microbubble activities, the initiating factor for BBB permeation [26,31].

> *"...focused ultrasound application following intravenous administration of preformed microbubbles has been demonstrated to generate localized and transient blood–brain barrier opening, resulting in delivery of various agents from chemotherapy drugs to antibodies into the brain."*

With a water-filled acoustic coupling device placed on the head of the rat, pulsed US (duration: 120 s; spatial–temporal peak pressure: 0.62 MPa; frequency: 0.4 MHz; pulse repetition frequency: 1 Hz; burst length: 10 ms) was applied to generate BBB disruption at a target site within the brain (e.g., the tumor) guided by MRI. The pulse repetition frequency and burst length were typical, but most previous studies used US with 10–40 s duration [1–10]. The acoustic pressure (0.62 MPa) is also on the higher side in the range of reported values [31]. It is worth noting that the detailed mechanistic basis for these parameters is not available. Thus, these may not be the optimal US parameters to generate BBB disruption without damage to the endothelium. This study does not distinguish whether the US-induced BBB disruption was caused by inertial cavitation or stable cavitation. Inertial cavitation is the inertia-dominated large oscillation and rapid collapse of microbubbles driven by an US field, and can cause damage to the endothelium of blood vessels [32,33].

■ Multifunctional MNPs

The researchers synthesized Fe(II,III) magnetite MNPs using the popular co-precipitation method by precipitating iron isotypes Fe^{2+} and Fe^{3+} salts under basic conditions. By varying the reaction time and temperature, three

different types of MNPs were obtained with different core sizes (MNP-1: 10.9 nm; MNP-2: 11.4 nm; and MNP-3: 12.3 nm). Slower base addition coupled with lower temperature for the nucleating step and longer time for crystal growth for MNP-3 formation led to its larger core sizes. Furthermore, MNP-3 has higher crystallinity and magnetic relaxivity (R_2 = 217 $mM^{-1}s^{-1}$), which is important for MT and acting as an MRI contrast agent. Through noncovalent interactions, the MNPs were then coated with a carboxylic acid-functionalized polyaniline polymer (SPAnH) to reduce aggregation and improve colloidal stability in water. The properties of the MNPs were compared with those of the commercially available Resovist® (R_2 = 115 $mM^{-1}s^{-1}$), which is prepared by forming Fe(II,III) crystals in the presence of a polysaccharide (carboxydextran). The hydrodynamic radii of Resovist (64 nm) is comparable with that of MNP-3 (83 nm) with similar surface charges (ζ potentials ~45 mV); therefore, these parameters most likely do not cause significant difference in cellular uptake. The major distinction between Resovist and MNP-3 is in their response to an external magnetic field. With its highly soluble carboxydextran coating, Resovist is very colloidally stable and does not readily precipitate when a magnetic field is applied. By contrast, MNP-3 easily came out of solution in a magnetic field, thus making it suitable for MT.

> "*This study explored a combined strategy ... and has achieved improved outcome of epirubucin delivery to tumors in the brain in a rat model.*"

Epirubicin is a cytotoxic anticancer agent with significant cardiac toxicity. Therefore, targeted delivery of epirubicin to a tumor can potentially enhance its chemotherapeutic effects while reducing its systemic toxicity. Covalently linked to MNP-3 through amidation with the carboxylic acids on the MNP-3 surface, up to 300 µg of epirubicin per mg of MNP-3 was attached, which was high. Following MNP incubation with the tumor cells, significant cellular uptake of the MNP-3 by endocytosis was observed, although it was unclear whether epirubicin was released from the nanoparticles and how the drug was distributed. Nevertheless, upon immobilization onto MNP-3, the cytotoxicity of epirubicin towards cancer cells was comparable with that of the free drug. When a magnet was applied, the toxicity significantly improved (IC_{50} 1.7 µg/ml with MT compared with 5.2 µg/ml without MT), presumably due to the increased local concentration of the drug-bearing MNPs.

■ Enhanced delivery of MNPs & epirubicin into the brain

Unable to produce a focused magnetic field, an inhomogeneous magnetic field to attract the MNPs to the targeted site was achieved by tilting a permanent magnet tied to the animal head at an angle to cover a portion of the brain. MRI showed that focused US alone increased local deposition of MNP-3 by 21.5% relative to the contralateral hemisphere in a normal brain. Subsequent application of MT increased MNP accumulation up to 244.6% after 6 h of MT, achieving a concentration of epirubicin 21.738 ± 3.477 ng/g of tissue measured by HPLC, a significant improvement on the 1.336 ± 1.182 ng/g with only US application. In animals with tumors, the epirubicin concentration in the tumor was increased to 11.982 ± 2.104 ng/g by US and MT, also a significant increase but only approximately 50% of that in a normal brain. The tumor volume increased over a 7-day period by 106 ± 24% compared with 313 ± 103% in controls. Compared with 18.3 days for the control group, the medium animal survival increased to 23 and 20 days with MNPs alone and US plus MNPs, respectively, and to 30 days for US plus MT plus MNPs. This suggested that both US and MT are essential to bestow beneficial therapeutic effects to brain tumors.

After crossing the BBB aided by US and MT, the MNPs reached the tumor cells, presumably through passive diffusion, and entered the cells by endocytosis. Although unexplored in the current study, agents that can actively target the tumor through cell-specific binding can be immobilized onto the MNPs. These multifunctional MNPs could potentially further enhance the drug concentration inside the tumor cells.

■ Mechanisms of US-induced BBB permeation & enhanced transport of MNPs

Although US-driven microbubble cavitation, which produces localized yet significant mechanical impacts such as shear stress [34–36], micro-streaming [37,38] and other mechanical forces [34–36,39,40], can generate BBB permeation, the detailed mechanisms of US-induced BBB permeation are not completely known.

Possibilities include free diffusion/passage through injured endothelium, enhanced transcellular passage and paracellular transport [41,42]. The outcome of transport depends on the exact mechanism of US-induced BBB permeation; for example, paracellular transport of hydrophilic molecules or agents relies on the TJ opening. Transcellular transport of hydrophobic drugs rely on their partition into the endothelial cell membrane [43], although recognition of these hydrophobic molecules by efflux pumps (e.g., P-gp) on the luminal surface of the endothelial cells limit their net transport into the brain [44].

In this study, the MNPs were taken up by the tumor cells after US and MT application, while in the control group the MNPs were contained in the vasculature. The increased intracellular uptake of the MNPs was due to endocytosis enhanced by the higher local concentration of the MNPs after US and MT, not sonoporation [45]. Transmission electron microscopy showed inter-endothelial clefts without obvious TJ complexes at the tumor site, but no evidence was provided in this study regarding the mechanisms of BBB permeation in either normal brain or tumors. The role of P-gp was not discussed, although epirubicin is a known substrate for P-gp. While it may be beyond the scope of this study, understanding of the exact mechanisms of the observed transport by US and MT, as well as a detailed assessment of tissue damage, are critical in determining the clinical translatability of the technique for delivery of chemotherapeutic drugs to treat brain tumors in a rational fashion.

Clinical translation

Although this study has demonstrated the successful application of MT combined with US application in brain tumor treatment, translation of the technology to the clinics still faces many challenges. As US-induced BBB permeation can last 4–12 h [1,41], the duration (e.g., 3–6 h) of MT necessary to achieve the enhancement in this study may be within the window of BBB permeation. However, whether this long duration is a limiting factor for clinical applicability is unclear. Another major issue is the distance between the magnet and the tumor site, which was less than 1 cm in the current rat model. The magnetic field declined sharply over distance and MT would be ineffective in deep tissues. Potential solutions include the usage of stronger super-conducting magnets as well as better engineering of the MNPs to improve their responses in magnetic fields.

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Bibliography

1 Hynynen K, Mcdannold N, Vykhodtseva N, Jolesz FA. Noninvasive MR imaging-guided focal opening of the blood–brain barrier in rabbits. *Radiology* 220(3), 640–646 (2001).

2 Hynynen K, Mcdannold N, Sheikov NA, Jolesz FA, Vykhodtseva N. Local and reversible blood–brain barrier disruption by noninvasive focused ultrasound at frequencies suitable for *trans*-skull sonications. *Neuroimage* 24(1), 12–20 (2005).

3 Kinoshita M, Mcdannold N, Jolesz FA, Hynynen K. Noninvasive localized delivery of herceptin to the mouse brain by MRI-guided focused ultrasound-induced blood–brain barrier disruption. *Proc. Natl Acad. Sci. USA* 103(31), 11719–11723 (2006).

4 Kinoshita M, Mcdannold N, Jolesz FA, Hynynen K. Targeted delivery of antibodies through the blood–brain barrier by MRI-guided focused ultrasound. *Biochem. Biophys. Res. Commun.* 340(4), 1085–1090 (2006).

5 Choi JJ, Wang S, Tung YS, Morrison B 3rd, Konofagou EE. Molecules of various pharmacologically-relevant sizes can cross the ultrasound-induced blood–brain barrier opening *in vivo*. *Ultrasound Med. Biol.* 36(1), 58–67 (2010).

6 Treat LH, Mcdannold N, Vykhodtseva N, Zhang Y, Tam K, Hynynen K. Targeted delivery of doxorubicin to the rat brain at therapeutic levels using MRI-guided focused ultrasound. *Int. J. Cancer* 121(4), 901–907 (2007).

7 Hynynen K. Macromolecular delivery across the blood–brain barrier. *Methods Mol. Biol.* 480, 175–185 (2009).

8 Xie F, Boska MD, Lof J, Uberti MG, Tsutsui JM, Porter TR. Effects of transcranial ultrasound and intravenous microbubbles on blood–brain barrier permeability in a large animal model. *Ultrasound Med. Biol.* 34(12), 2028–2034 (2008).

9 Liu HL, Hua MY, Chen PY *et al.* Blood–brain barrier disruption with focused ultrasound enhances delivery of chemotherapeutic drugs for glioblastoma treatment. *Radiology* 255(2), 415–425 (2010).

10 Liu Y, Paliwal S, Bankiewicz KS *et al.* Ultrasound-enhanced drug transport and distribution in the brain. *AAPS PharmSciTech* 11(3), 1005–1017 (2010).

11 Mcdannold N, Clement GT, Black P, Jolesz F, Hynynen K Transcranial magnetic resonance imaging-guided focused ultrasound surgery of brain tumors: initial findings in 3 patients. *Neurosurgery* 66(2), 323–332 (2010).

12 Liu HL, Hua MY, Yang HW *et al.* Magnetic resonance monitoring of focused ultrasound/magnetic nanoparticle targeting delivery of therapeutic agents to the brain. *Proc. Natl Acad. Sci. USA* 107(34), 15205–15210 (2010).

13 Schouten LJ, Rutten J, Huveneers HA, Twijnstra A. Incidence of brain metastases in a cohort of patients with carcinoma of the breast, colon, kidney, and lung and melanoma. *Cancer* 94(10), 2698–2705 (2002).

14 Pardridge WM. The blood–brain barrier: bottleneck in brain drug development. *NeuroRx* 2(1), 3–14 (2005).

15 Tosoni A, Ermani M, Brandes AA. The pathogenesis and treatment of brain metastases: a comprehensive review. *Crit. Rev. Oncol. Hematol.* 52(3), 199–215 (2004).

16 Pardridge WM. Drug and gene delivery to the brain: the vascular route. *Neuron* 36(4), 555–558 (2002).

17 Cecchelli R, Berezowski V, Lundquist S *et al.* Modelling of the blood–brain barrier in drug discovery and development. *Nat. Rev. Drug Discov.* 6(8), 650–661 (2007).

18 Juillerat-Jeanneret L. The targeted delivery of cancer drugs across the blood–brain barrier: chemical modifications of drugs or drug-nanoparticles? *Drug Discov. Today* 13(23–24), 1099–1106 (2008).

19 Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ. Structure and function of the blood–brain barrier. *Neurobiol. Dis.* 37(1), 13–25 (2010).

20 Pardridge WM. Blood–brain barrier delivery. *Drug Discov. Today* 12(1–2), 54–61 (2007).

21 Pardridge WM. Re-engineering biopharmaceuticals for delivery to brain with molecular Trojan horses. *Bioconjug. Chem.* 19(7), 1327–1338 (2008).

22 Jahnke K, Kraemer DF, Knight KR *et al.* Intraarterial chemotherapy and osmotic blood–brain barrier disruption for patients with embryonal and germ cell tumors of the central nervous system. *Cancer* 112(3), 581–588 (2008).

23 Doolittle ND, Abrey LE, Bleyer WA *et al.* New frontiers in translational research in neuro-oncology and the blood–brain barrier: report of the tenth annual blood–brain barrier disruption consortium meeting. *Clin. Cancer Res.* 11(2 Pt 1), 421–428 (2005).

24 Vogelbaum MA. Convection enhanced delivery for treating brain tumors and selected neurological disorders: symposium review. *J. Neurooncol.* 83(1), 97–109 (2007).

25 Bidros DS, Liu JK, Vogelbaum MA. Future of convection-enhanced delivery in the treatment of brain tumors. *Future Oncol.* 6(1), 117–125 (2010).

26 Mcdannold N, Vykhodtseva N, Hynynen K. Targeted disruption of the blood–brain barrier with focused ultrasound: association with cavitation activity. *Phys. Med. Biol.* 51(4), 793–807 (2006).

27 Hynynen K, Clement GT, Mcdannold N *et al.* 500-element ultrasound phased array system for noninvasive focal surgery of the brain: a preliminary rabbit study with *ex vivo* human skulls. *Magn. Reson. Med.* 52(1), 100–107 (2004).

28 Choi JJ, Pernot M, Small AS, Konofagou EE. Noninvasive, transcranial and localized opening of the blood–brain barrier using focused ultrasound in mice. *Ultrasound Med. Biol.* 33(1), 95–104 (2007).

29 Marquet F, Pernot M, Aubry JF *et al.* Non-invasive transcranial ultrasound therapy based on a 3D CT scan: protocol validation and *in vitro* results. *Phys. Med. Biol.* 54(9), 2597–2613 (2009).

30 Deng CX. Targeted drug delivery across the blood–brain barrier using ultrasound technique. *Ther. Deliv.* 1(6) 819–848 (2010).

31 Mcdannold N, Vykhodtseva N, Hynynen K. Effects of acoustic parameters and ultrasound contrast agent dose on focused-ultrasound induced blood–brain barrier disruption. *Ultrasound Med. Biol.* 34(6), 930–937 (2008).

32 Tu J, Hwang JH, Matula TJ, Brayman AA, Crum LA. Intravascular inertial cavitation activity detection and quantification *in vivo* with optison. *Ultrasound Med. Biol.* 32(10), 1601–1609 (2006).

33 Price RJ, Skyba DM, Kaul S, Skalak TC. Delivery of colloidal particles and red blood cells to tissue through microvessel ruptures created by targeted microbubble destruction with ultrasound. *Circulation* 98(13), 1264–1267 (1998).

34 Wu J. Shear stress in cells generated by ultrasound. *Prog. Biophys. Mol. Biol.* 93(1–3), 363–373 (2007).

35 Vanbavel E. Effects of shear stress on endothelial cells: possible relevance for ultrasound applications. *Prog. Biophys. Mol. Biol.* 93(1–3), 374–383 (2007).

36 Marmottant P, Hilgenfeldt S. Controlled vesicle deformation and lysis by single oscillating bubbles. *Nature* 423(6936), 153–156 (2003).

37 Van Wamel A, Kooiman K, Harteveld M *et al.* Vibrating microbubbles poking individual cells: drug transfer into cells via sonoporation. *J. Control. Release* 112(2), 149–155 (2006).

38 Collis J, Manasseh R, Liovic P *et al.* Cavitation microstreaming and stress fields created by microbubbles. *Ultrasonics* 50(2), 273–279 (2010).

39 Pecha R, Gompf B. Microimplosions: cavitation collapse and shock wave emission on a nanosecond time scale. *Phys. Rev. Lett.* 84(6), 1328–1330 (2000).

40 Ohl CD, Arora M, Ikink R *et al.* Sonoporation from jetting cavitation bubbles. *Biophys. J.* 91(11), 4285–4295 (2006).

41 Sheikov N, Mcdannold N, Sharma S, Hynynen K. Effect of focused ultrasound applied with an ultrasound contrast agent on the tight junctional integrity of the brain microvascular endothelium. *Ultrasound Med. Biol.* 34(7), 1093–1104 (2008).

42 Sheikov N, Mcdannold N, Vykhodtseva N, Jolesz F, Hynynen K. Cellular mechanisms of the blood–brain barrier opening induced by ultrasound in presence of microbubbles. *Ultrasound Med. Biol.* 30(7), 979–989 (2004).

43 Jong A, Huang SH. Blood–brain barrier drug discovery for central nervous system infections. *Curr. Drug Targets Infect. Disord.* 5(1), 65–72 (2005).

44 Sugimoto H, Hirabayashi H, Kimura Y, Furuta A, Amano N, Moriwaki T. Quantitative investigation of the impact of P-glycoprotein inhibition on drug transport across blood–brain barrier in rats. *Drug Metab. Dispos.* 39(1), 8-14 (2011)

45 Deng CX. Sonoporation and gene delivery. In: *Therapeutic Ultrasound: Mechanisms to Applications.* Frenkel V (Ed.). Nova Science Publishers, NY, USA (2010).