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The kinetics of blood brain barrier permeability and targeted doxorubicin delivery into brain induced by focused ultrasound

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Abstract

Focused ultrasound (FUS) combined with a circulating microbubble agent is a promising strategy to non-invasively disrupt the blood-brain barrier (BBB) and could enable targeted delivery of therapeutics that normally do not leave the brain vasculature. This study investigated the kinetics of the BBB permeability using dynamic contrast-enhanced MRI (DCE-MRI) and the resulting payload of the chemotherapy agent, doxorubicin (DOX). We also investigated how the disruption and drug delivery were affected by a double sonication (DS) with two different time intervals (10 or 120 min). Two locations were sonicated transcranially in one hemisphere of the brain in 20 rats using a 690 kHz FUS transducer; the other hemisphere served as a control. For BBB disruption, 10 ms bursts were applied at 1 Hz for 60 s and combined with IV injection of a microbubble ultrasound contrast agent (Definity; 10 μ l/kg). DOX was injected immediately after the second location was sonicated. The transfer coefficient (K_{trans}) for an MRI contrast agent (Gd-DTPA) was estimated serially at 4–5 time points ranging from 30 min to 7.5 hr after sonication using DCE-MRI. After a single sonication (SS), the mean K_{trans} was $0.0142 \pm 0.006 \text{ min}^{-1}$ at 30 min and was two or more orders of magnitude higher than the non-sonicated targets. It decreased exponentially as a function of time with an estimated half-life of 2.22 hr (95% confidence intervals (CI): 1.06–3.39 hr). Adding a second sonication increased K_{trans} , and with a 120 min interval between sonications, prolonged the duration of the BBB disruption. Mean K_{trans} estimates of 0.0205 (CI: 0.016–0.025) and 0.0216 (CI: 0.013–0.030) min^{-1} were achieved after DS with 10 and 120 min delays, respectively. The half-life of the K_{trans} decay that occurred as the barrier was restored was 1.8 hr (CI: 1.20–2.41 hr) for a 10 min interval between sonications and increased to 3.34 hr (CI: 0.84–5.84 hr) for a 120 min interval. DOX concentrations were significantly greater than in the non-sonicated brain for all experimental groups ($p < 0.0001$), and 1.5-fold higher for DS with a 10 min interval between sonications. A linear correlation was found between the DOX concentration achieved and the K_{trans} measured at 30 min after sonication ($R: 0.7$). These data suggest that one may be able to use Gd-DTPA as a surrogate tracer to estimate DOX delivery to the brain after FUS-induced BBB disruption. The results of this study provide information needed to take into account the dynamics BBB disruption over time after FUS.

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Keywords

Focused ultrasound; Blood-brain barrier disruption; Pharmacokinetic model; Dynamic contrast enhanced MRI

INTRODUCTION

It has been demonstrated that focused ultrasound (FUS) bursts combined with a circulating microbubble agent can induce localized blood-brain barrier (BBB) disruption (BBBD) [1–3]. Since the microbubbles are restricted to the vasculature, mechanical effects produced by the microbubble dynamics are localized to blood vessels and result in temporary BBBD. There exists a range of acoustic pressure amplitudes that produce no or only minimal vascular injury or other effects in addition to the disruption [4–6]. This technique has been shown to facilitate the targeted delivery of circulating therapeutic agents from the vasculature to the brain parenchyma that are normally restricted due to the BBB [7–10]. Electron microscopy study indicates that the FUS-induced BBBD enables passive transport through widened tight junctions (TJs) as well as active transport [1, 3, 11–13]. With this approach, substances with a wide range of molecular sizes can pass through the BBB, including chemotherapy agents [8–10, 14] and antibodies [7, 15, 16].

The potential impact of FUS-induced BBBD on drug delivery in the brain would increase if one could predict or measure how much drug is delivered to each time point in the target volume. The most straightforward means to achieve this would be through the use of drugs that are labelled with a contrast agent for medical imaging, enabling direct measurements of drug concentration *in vivo*. However, such an approach may not be feasible in most cases due to the expense involved in getting regulatory approval for each labelled drug, and the labelling itself may result in altered drug delivery and action. From this standpoint, a more desirable approach would be that currently-approved medical imaging contrast agent could be used as a surrogate for the drug delivery. If one co-injects the surrogate and the drug, they could estimate the drug concentration based on estimates of the contrast agent concentration. This approach has been used for convection-enhanced drug delivery in the brain [17, 18], and prior work has shown that the MRI signal enhancement after contrast injection correlates with the amount of drug delivered to the brain [7, 10, 19] after FUS-induced BBBD. As the amount of drug delivered depends on drug properties (molecular weight, plasma half-life, charge, lipophilicity, etc.) and several other factors, the relationship between the contrast and drug delivery may need to be evaluated for each drug.

A number of studies have been performed to evaluate the magnitude and duration of FUS-induced BBBD under different conditions. The degree of BBBD induced by FUS can be affected by exposure parameters such as ultrasound frequency, acoustic pressure amplitude, pulse length, pulse repetition frequency, sonication duration, and the dose and size of the microbubble-based ultrasound contrast agent (USCA) [6, 20–25]. In many cases, the degree and time course of the BBBD has been evaluated for different ultrasound parameters using signal intensity changes of contrast-enhanced magnetic resonance imaging (MRI) [1]. Several studies have shown that the BBB, as evidenced by a lack of contrast enhancement in MRI, is largely restored within few hours after sonication [1, 2]. An immunoelectron microscopy study [13] also showed that the numbers of several TJs-specific proteins were significantly reduced for four hours after sonication and appeared completely restored between 6 hrs and 24 hrs after sonication.

To predict or model how much drug will be delivered to the brain, one needs to characterize the permeability of the disrupted BBB. The permeability of contrast agents can be

characterized using dynamic contrast enhanced (DCE) imaging and pharmacokinetic models [26]. Such analysis was performed previously in mice approximately at 1–2 hr after FUS-induced BBB [27, 28]. However, it is known that the barrier function begins to be restored fairly quickly after sonication [1, 2], and to fully utilize such models the dynamics of this restoration needs to be considered. Thus, in this work, we performed repeated contrast injections and DCE analysis to characterize the permeability changes as a function of time after BBB. We also confirmed that the transfer coefficient estimated for an MRI contrast agent describing the transport from the vasculature to the brain with such modeling is predictive of the delivery of a chemotherapy agent, Doxorubicin hydrochloride (DOX). Finally, we evaluated how these dynamics are affected by additional sonication and whether we could safely use such additional exposures to extend the magnitude of the BBB and the time at which the BBB is disrupted.

MATERIALS AND METHODS

Animals

The experiments were approved by our institutional animal care and use committee. Twenty male Sprague-Dawley rats (Charles River Laboratories, Boston, MA; weight: 250–350 g) were used for this study. The animals were divided into four groups based on treatment protocol (Table 1). Before the sonications, the animals were anesthetized with a mix of 80 mg/kg of ketamine (Aveco Co., Inc., Fort Dodge, IA) and 10 mg/kg of xylazine (Lloyd Laboratories, Shenandoah, IA) by intraperitoneal injection, the hair on the scalp was removed with clippers and depilatory cream (Nair, Church & Dwight Co., Inc., Princeton, NJ), and a catheter was placed in the tail vein. Body temperature was maintained throughout the experiment with a heated water pad.

Equipment

The ultrasound system and experimental setup were the same as we used in our previous study [1]. A single-element, spherically curved, piezoelectric transducer with a diameter of 100 mm and radius of curvature of 80 mm operating at 690 kHz (manufactured in-house) generated the ultrasound field. The absolute and relative peak negative pressure amplitudes were measured in a water tank with a calibrated 0.5-mm diameter membrane hydrophone (Marconi, Chelmsford, UK) and a 0.075-mm diameter needle hydrophone (Precision Acoustics, Dorchester, UK). The exposure conditions throughout the present study are given in peak rarefactional focal pressure (PRFP) amplitude. The half-maximum pressure amplitude width and length of the focal region for this transducer were 2.3 and 14 mm, respectively [1]. The transducer was immersed in a tank of degassed water and mounted on an MR-compatible positioning system. The experiments were performed in a clinical 3T MRI scanner (General Electric Healthcare, Milwaukee, WI). MRI was used for image guidance and BBB evaluation. The imaging was performed using a 7.5 cm diameter transmit/receive surface coil (constructed in-house).

The transducer was driven by a signal generated by an arbitrary waveform generator (Model 395, Wavetek Inc., San Diego, CA) and an RF amplifier (Model 240L, ENI Inc., Rochester, NY). The electrical impedance of the transducer was matched to the output impedance of the amplifier using an external inductor-capacitor tuning network. The electrical power was monitored with a power meter (Model E4419B, Agilent, Santa Clara, CA) and a dual-directional coupler (Werlatone, Patterson, NY). The transducer efficiency was measured with a radiation force balance consisting of an absorbing brush attached to a digital scale.

Sonications

Burst sonications (10 ms bursts applied at 1 Hz for 60 s) at acoustic power levels ranging from 0.34–0.59 W were delivered transcranially into the brain of the rat, which laid in the supine position on the sonication system (Fig. 1A). These power levels corresponded to PRFP amplitudes in water of 0.6 – 0.8 MPa. The initial exposure levels used were obtained from a prior safety study with this transducer in rats [29].

Two locations were targeted (5 mm apart from each other) in the right hemisphere of each animal. The targets were located 2.5 mm lateral to the midline and 5 mm deep from the dorsal brain surface. They were centered 2.5 mm anterior and posterior to the bregma, in the right striatum and hippocampus, respectively. The striatum was of interest to us because of its involvement with motor function and its potential for drug therapies for Huntington's and Parkinson's disease. The hippocampus was of interest to us as a potential target for Alzheimer's disease. Control locations were selected at the same anatomical structures in the left hemisphere. Each sonication was applied synchronous to an IV bolus injection of USCA (Definity™, Lantheus Medical Imaging, N. Billerica, MA). Definity™ consists of a C₃F₈ gas encapsulated by an outer phospholipid shell. Immediately after activation, the suspension contains approximately 1.2×10^{10} microbubbles/mL with a mean diameter range of 1.1 μm –3.3 μm . For the current study, the solution was diluted 10 times in PBS and was injected IV at a dosage of 10 $\mu\text{l/kg}$, the recommended dose for clinical use with ultrasound imaging. The administration of Definity™ was followed by an injection of 0.2 mL normal saline (0.9% NaCl). Sonication of the individual locations was spaced at least 2 min apart to allow the agent to mostly clear from the vascular.

In experimental groups 3 and 4, each location was sonicated twice, with either a 10 or 120 min interval between the sonications. An interval of 10 min was selected to mimic a potential patient treatment, where it may be determined that the BBBD achieved by an initial sonication was insufficient. This time is also long enough for the microbubbles to clear the circulation and for acute physiological responses induced by the sonication such as vasospasm [30] to be resolved. The 120 min interval was selected to evaluate whether the duration of the BBBD could be extended. It is a duration where the BBBD is expected to be largely resolved [1, 2] and enabled us to evaluate whether further disruption is possible at the same time that the barrier function is being restored.

Delivery of DOX and trypan blue dye

Doxorubicin hydrochloride (DOX; Novaplus, Irving, TX) was selected as a model drug to compare with the permeability characterization in this study. DOX (molecular weight: 579.98 Da) was selected because it is one of the most widely-used chemotherapeutic agents available, but it does not cross the intact BBB [31]. In addition, DOX could be extracted from the harvested tissue samples and readily quantified with fluorometric analysis [10, 32]. DOX was administered IV at a dose of 5.67 mg/kg immediately after the second location was sonicated (Fig. 1B). This dose was selected to be similar to what has been used clinically. For animals sonicated twice, DOX was administered after the first set of sonications. Note that due to the short plasma half-life of DOX (5.3 min) [33], the second sonication was not expected to change the amount of drug delivered to the brain for a 120 min interval. For this group, when DOX tissue concentrations were compared to MRI measurements, we used the MRI measurements obtained after the first sonication.

To confirm successful BBBD and mark the targeted areas for tissue extraction, a vital dye, trypan blue (MP Biomedicals, Solon, OH) was administered after the second sonication. The agent was prepared for each experiment as described previously [34]. The agent was

dissolved in 0.45 % NaCl and then boiled and filtered to avoid formation of microcrystals. The solution was administered IV at a dose of 0.1 g trypan blue per kg of body weight.

Magnetic resonance imaging

Most MRI procedures were similar to those described previously [1]. Before the rat experiments, the target location of the FUS beam in the MRI coordinate-space was visualized by imaging temperature changes induced in a silicone phantom with a T1-weighted fast spin echo (FSE) sequence. Then, the animal was placed supine on the focused ultrasound system with the dorsal surface of the head centered in the MRI coil (Fig. 1A). Standard MR imaging sequences were used to select the brain targets, characterize the BBB, and evaluate the brain for tissue damage. The sequences used and imaging parameters are listed in Table 2. T2-weighted FSE imaging was used to select the targets. After sonication, DCE imaging was performed at different time points to evaluate the permeability of the BBB (see below). For this imaging, we used the MRI contrast agent gadopentetate dimeglumine (Gd-DTPA) (Magnevist®, Berlex Laboratories, Inc., Wayne, NJ, USA; molecular weight: 938 Da; plasma half-life in rats: 13 min [35]) administered as a bolus injection at a dose of 0.125 mmol per kg of body weight. After DCEMRI acquisition, T1-weighted FSE images were acquired to further visualize the BBB, and T2*-weighted spoiled gradient echo (SPGR) images were acquired to evaluate whether extensive petechiae were produced.

Dynamic contrast-enhanced imaging

The Gd-DTPA concentration was estimated as a function of time at each image voxel with the following equation:

$$C(t) = \left(\frac{1}{T_1(t)} - \frac{1}{T_{10}} \right) / \mathfrak{R}_{gd} \quad (1)$$

where T_{10} is the pre-contrast T_1 value and $\mathfrak{R}_{gd}$ is the relaxivity of Gd-DTPA, which is $4.39 \text{ s}^{-1} \text{ mM}^{-1}$ [36]. Maps of T_{10} were generated by fitting the signal intensity of an SPGR sequence acquired at multiple flip angles as described elsewhere [37]. $T_1(t)$ was estimated using a SPGR sequence with the following expression:

$$\frac{1}{T_1(t)} = -\frac{1}{TR} * \log \left[\frac{1-A}{1-\cos\alpha * A} \right], \text{ where } A = \frac{1 - \exp(-TR/T_{10})}{1 - \cos\alpha * \exp(-TR/T_{10})} * \frac{S(t)}{S(0)} \quad (2)$$

where α and TR are the flip angle and repetition time of the SPGR sequence, respectively. $S(t)$ is the signal intensity of the sequence as function of time, with $S(0)$ indicating the signal intensity before the contrast injection.

The Gd-DTPA concentration was fit to a modified Tofts-Kermode model [26] that takes into account the presence of separate extracellular and intravascular compartments [38]. The tissue concentration of Gd-DTPA ($C_t(t)$), measured with equation (1), was modeled with the following equation:

$$C_t(t) = K_{trans} \int_0^t C_p(\tau) e^{\frac{K_e}{v_e}(t-\tau)} d\tau + v_p \cdot C_p(t), \quad (3)$$

where v_e and v_p are the fraction of the extracellular-extravascular space (EES) and blood plasma volume, respectively, that contain contrast agent, and $C_p(t)$ is the plasma concentration of Gd-DTPA. K_{trans} and K_e are rate constants describing the transfer of contrast agent between blood plasma and the EES, and from the EES back to the blood.

plasma, respectively. Equation (3) was simplified by neglecting the exponential term, because $K_e v_e$ or $(t-\tau)$ is small [37].

$$C_t(t) = K_{trans} \int_0^t C_p(\tau) d\tau + v_p \cdot C_p(t) \quad (4)$$

The K_{trans} and v_p values were extracted from fits of the data the equation (4) by using least squares regression with TOPPCAT [37]. $C_p(t)$, the concentration vs. time curve of Gd-DTPA in plasma, was chosen from a region of interest in a vascular unit (ophthalmic artery or transverse sinus) and was adjusted for a 45% capillary hematocrit level [37].

For the DCE imaging, eight pre-contrast sets of SPGR images were acquired. Then, a bolus of Gd-DTPA was administered IV and an additional 62 sets of SPGR images were acquired with a temporal resolution of 7.6 seconds for 8 min. Identical slice locations were used for T1 mapping and DCE-MRI. The procedure (T1 mapping, Gd-DTPA injection, and DCE-MRI) was repeated 4–5 times after the sonications to characterize the BBB permeability as a function of time. The time interval between measurements was at least an hour to avoid excessive Gd-DTPA accumulation. The animals remained in place within the MRI over the entire experiment.

DOX extraction

The concentration of DOX in the tissue was quantified using fluorometric analysis. For these measurements, tissue samples were extracted from the brain following a protocol adapted from a previous study [10, 32]. Approximately 16 hr after the last sonication, the animals were deeply anaesthetized with ketamine/xylazine and sacrificed. To flush DOX from the cerebral vasculature, the brain was transcardially perfused with normal saline (0.9% NaCl, 250 mL). Then, the brain was removed and small tissue volumes (approximately 30 mg) identified by trypan blue staining were harvested along with their contralateral counterparts, which served as controls. The samples were soaked in acidified ethanol (50% ethanol in 0.3N HCl). After homogenization with a tissue blender (Next Advance, Averill Park, NY) and refrigeration for 24 hr at 4°C, the samples were centrifuged at 16,000 g for 25 min. The fluorescent intensity of the supernatant was measured using a benchtop fluorometer (VersaFluor; Bio-Rad Laboratories, Hercules, CA; Ex/Em: 480/590 nm). The concentration of DOX was quantified by linear regression and a standard curve derived from eight serial concentrations. The concentration of DOX for each tissue sample was determined by taking the average of at least of three readings.

Histology

To evaluate the histological effects of the ultrasound protocol used in this study, three of the animals were sacrificed four hours after the last sonication. DOX or trypan blue was not administered in the animals used for the histology study. The animal was deeply anesthetized with ketamine/xylazine, sacrificed, and the brain fixed via transcardial perfusion (0.9% NaCl, 100 mL; 10% buffered formalin phosphate, 250 mL). The brain was then removed, embedded in paraffin, and serially sectioned at 5 μ m sections in the axial plane (perpendicular to the direction of ultrasound beam propagation). Every 50th section (250 μ m apart) was stained with hematoxylin and eosin (H&E). The author who evaluated the histology was blind to the FUS exposure parameters.

Statistical analysis

MRI and DOX measurements for the sonicated and contralateral (control) volumes were compared using a two-tailed paired Student's t test. Values of $p < 0.05$ were considered statistically significant. K_{trans} measurements as a function of time were fit to the following equation:

$$K_{trans}(t) = K_{trans}^0 e^{-t/R}, \quad (5)$$

where K_{trans}^0 is the estimated K_{trans} value immediately after sonication and R is the decay constant of K_{trans} . These coefficients, along with 95% confidence intervals (CI), were found using non-linear least squares regression in Matlab (Mathworks, Natick, MA). Additional analyses included least-squares linear regression and calculation of correlation coefficients. K_{trans} values presented are mean values ($\pm$ standard deviation) obtained in a 0.75×0.75 mm region of interest.

RESULTS

BBBD and kinetics of BBB permeability changes

In initial experiments (group 1 in Table 1), sonications with PRFP amplitudes between 0.6 and 0.8 MPa were investigated in eight animals. Targets were centered 2.5 mm anterior and posterior to the bregma. Similar to previous findings [10], we found that the posterior targets appeared to be more sensitive than the anterior ones. FUS-induced BBBD, as verified with contrast-enhanced T1-weighted MRI, was found to be possible in these animals without evidence of damage on T2*-weighted imaging at pressure amplitudes of 0.68 and 0.72 MPa for the posterior and anterior targets, respectively. These pressure amplitudes were used in subsequent experiments. Figure 2 shows examples where contrast enhancement was observed at the two locations without hypointense spots detected in the T2*-weighted MR images. The presence of hypointense spots indicate the production of extensive petechiae and were evident at higher PRFP amplitudes.

The kinetics of the FUS-induced BBB permeability was evaluated for 4.5 hours after a single sonication (SS) using serial DCE-MRI measurements in six animals (group 2 in Table 1). Fig. 3 shows examples for the two animals that had locations with the largest and smallest K_{trans} measurements in this group. Following sonication, increased permeability at the targeted locations was evident by signal enhancement in the T1-weighted images (Fig. 3A, E) and by elevated K_{trans} values (Fig. 3 B, F). No hypointense regions were observed in the T2*-weighted imaging in 11/12 of the targeted locations in this group (Fig. 3D, H); the single location that was hypointense after sonication was excluded from the analysis. At the first measurements (30 min after sonication), K_{trans} varied from 0.0086 to 0.0232 min^{-1} (Fig. 3C, G). It then decreased as a function of time, while the K_{trans} measurements in the contralateral hemisphere (control) were near zero at each time point. The mean K_{trans} value for the 11 BBB disrupted areas was $0.0142 \pm 0.006 \text{ min}^{-1}$ at 30 min after sonication (Fig. 4A). Fitting the data to an exponential decay (equation (5)) yielded an estimated half-life of the K_{trans} decay of 2.2 hr. Table 3 shows the results of this regression for the experimental groups.

To evaluate the effects of repeated sonication on the BBBD kinetics, a second sonication was applied after a 10 (Fig. 4B) or 120 min (Fig. 4C) interval (groups 3 and 4 in Table 1). After double sonication (DS) with a 10 min interval, a K_{trans} of $0.0181 \pm 0.004 \text{ min}^{-1}$ was found 30 min after sonication, which was 28% higher than that found for a single sonication (SS). Regression of the exponential decay resulted in an estimated half-life of 1.8 hr, similar to the SS experiments. For the animals where a second sonication was applied after 120 min, the mean K_{trans} increased from a value of 0.0107 ± 0.003 after the first sonication to $0.0179 \pm 0.008 \text{ min}^{-1}$ at 60 min after the second. This value was significantly increased compared to the measurement obtained 30 min before the second sonication ($p < 0.05$). It then decreased exponentially with an estimated half-life of 3.3 hr.

DOX delivery

In all animals, DOX was administered after the second location was sonicated. A good correlation ($R=0.7$) was observed between K_{trans} measurements obtained 30 min after the sonications and the concentration of DOX at measured approximately 16 hours later (Fig. 5). Fig. 6 shows the mean concentration of DOX for a SS and for DS with 10 and 120 min intervals between sonications. In the SS group, the DOX concentration increased by 101% compared to control locations. DS with 10 min interval resulted in a 170% increase, and the DOX concentration was significantly higher than for the other groups ($p<0.05$). As expected based on DOX's short plasma half-life, the drug concentration achieved for the DS group with a 120 min interval was not significantly different than the SS group ($p=0.42$).

Histology

Histological examination was performed on one brain from each experimental group (SS, DS with 10 and 120 min intervals). Overall, in each case most of the sonicated region appeared unaffected in the H&E stained sections. The only effect of note was a small number of tiny clusters of extravasated erythrocytes (petechiae) that were scattered throughout the sonicated region. Between 0–10 clusters were found over entire sonicated region in each examined histological section. Figure 7 shows examples of no evident effects (Fig. 7A and E) and of the largest effects observed for the three experimental groups (Fig. 7B–D and F–H). As can be seen, the brain parenchyma and the neurons appear unaffected, and the only evident effect were the tiny (dimensions: 100 μm or less) petechiae. The number of these petechiae was similar for each of the three groups.

DISCUSSION

This study characterized the permeability of the rat brain to Gd-DTPA after BBB disruption induced by FUS and microbubbles and how it changed over time as the barrier was restored. This contrast agent, which normally does not enter the brain parenchyma from the bloodstream, could potentially be used as a surrogate marker for drug delivery. By understanding how the barrier recovers over time and the relationship between the delivery of the contrast agent and DOX, one could potentially use DCE-MRI acquired immediately after sonication to predict the amount of DOX that will be delivered.

The rate constant, K_{trans} measured at 30 min after a single sonication at the different sonicated spots varied between 0.0086 and 0.0232 min^{-1} (mean: $0.0142 \pm 0.0058 \text{ min}^{-1}$; Figs. 3 and 4A), which is within the range measured previously at 1–2 hr after sonication in mice [27, 28]. The large variation that was observed here was likely due to differences in skull thickness [39] and angle of incidence between the FUS beam and the skull surface for the different targets, and to the presence of standing waves within the intact skull cavity [40]. These differences likely added variation to the peak PRFP amplitude achieved at the focal point. However, despite this variation, the decay rate for K_{trans} was similar for the different animals. This decay rate and the estimated half-life (approximately 2–3 hours), are consistent with previous work that evaluated the duration of FUS-induced BBBD using MRI signal enhancement [1, 2] and through enumeration of tight junction proteins in the electron microscopy studies [13].

We also found that the permeability could be increased and prolonged with a second sonication. For both a 10 and 120 min delay between sonications, the measured K_{trans} increased by 26–28% on average compared to a single sonication, and with a 120 min delay the half-life of the K_{trans} decay towards baseline increased by over an hour. The ability of a second sonication to increase the BBB permeability is consistent with an earlier study that evaluated the delivery of Evans blue [41]. However, unlike in that work, we did not observe

tissue damage (tissue vacuolation) or any other increased tissue effects after the second sonication. These results are also consistent with experiments that have demonstrated that longer sonication produces BBBB with a larger magnitude [25].

The ability to increase the BBBB permeability with a second sonication could be used to increase the amount of drug delivered to the target region. This ability was shown with a significant improvement (1.5-fold) in DOX delivery in the animals where a DS was applied with a 10 min delay. More effective DOX delivery might be achieved using a shorter interval since the plasma half-life of DOX is only 5.3 min [33]. While DOX was not an appropriate drug to test with a 120 min interval due to its short half-life, the fact that we could increase the magnitude of BBB permeability and its duration after two hours (Fig. 4C) suggests that additional sonication after barrier is partially restored could be a useful strategy to enhance the delivery of agents with a long plasma half-life. Future work will be necessary to confirm whether that is the case.

The DOX concentration measured in the brain after sonication was linearly correlated with K_{trans} , as expected from equation (4). DOX is one of the most widely used chemotherapeutic agents, and it demonstrates efficacy for many types of cancer, including lymphoma [42–44], leukemia [45], and solid tumors [42, 45–48]. While it is cytotoxic for brain tumors in vitro [49], it has not been effective clinically, presumably in large part because of the BBB and other issues with effective delivery [31]. We are not aware of data relating DOX concentrations in brain tumors to clinical outcomes, but others have reported that intratumoral DOX concentrations of 819 ± 482 ng/g tumor (mean $\pm$ SD) were strongly correlated with a partial or complete response in 39% of breast cancer patients [50]. With the sonication parameters used in this study, this concentration was only achieved in targeted brain locations only for the group of DS with 10 min interval. For a single sonication, we can estimate based on the data shown in Fig. 5 that we would need to induce a K_{trans} value of 0.017 min^{-1} to reach this concentration, higher than what we were able to achieve. If this information were measured during a clinical treatment, it would suggest that we would need an additional sonication to reach this concentration.

For a single sonication, K_{trans} values of approximately 0.22 min^{-1} and higher were associated with hypointense spots in T2*-weighted imaging (Fig. 5). Such spots are indicative of extensive red blood cell extravasation and were avoided in all but one animal where serial K_{trans} measurements were obtained. We did find a few minor erythrocyte extravasations in histology as a result of these sonications. The presence of such extravasations suggests that a few capillaries were damaged by the sonications, which may have affected the magnitude and duration of the BBBB compared to sonications that did not produce such effects. Future work with a wider range of pressure amplitudes and more extensive histologic examination is needed to evaluate the influence of these tiny petechiae. We should note that the minor extravasations found in this study are expected to be resolved over time. Previous studies have demonstrated that they are almost completely removed four weeks after sonication [51].

Overall, the results from this study have characterized the kinetics of the BBB permeability using DCE-MRI and related them to measured tissue concentrations of DOX for a SS and DS. The permeability measured for a surrogate tracer and the drug concentrations showed a strong linear correlation. With this kinetic information of the BBB permeability, the pharmacokinetic model in equation (4) can be modified to take into account the parameters that reflect the closing of the BBB over time. We hope to use such pharmacokinetic modeling along with FUS-induced BBBB to develop a method for image-guided drug delivery that can estimate the amount of drug that will be delivered to each time point in the tissue and to optimize the drug infusion rate.

More information will be needed to create such a model. Most notably, future work is necessary to evaluate agents with different properties (molecular weight, plasma half-life, charge, lipophilicity, etc.) and different administration protocols (bolus, infusion) to understand under which circumstances such prediction is possible. Tests in tumors or other disease models will also be necessary to understand whether results from normal brain can be translated to situations where the vasculature is abnormal. In addition, the rate at which drugs are cleared from the brain should also be evaluated and included in the model. Finally, additional sonication parameters should be tested to determine whether they have an impact on how quickly the barrier recovers after FUS-induced BBBD.

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Figure 1.

A. Schematic of the experimental set-up used to induce BBB disruption in rats using FUS and microbubbles under MRI guidance. B. Experimental time line. The sonications were performed at two locations in one hemisphere in each rat. Each 60 s sonication started immediately after the microbubble injection. DOX and Trypan blue were administered immediately after the second location was sonicated, and then MRI was performed to characterize the disruption and to detect any damage. In some rats, each location was sonicated twice with either a 10 or 120 min interval between sonications. For double sonication with a 10 min interval (DS, 10 min), the second sonication was applied at right after the DOX and Trypan blue injections; with a 120 min interval (DS, 120 min), it was applied after the first MRI acquisition. The MRI protocol was repeated 4–5 times up to 7.5 hours after the last sonication. The rats were sacrificed at either 4 or 16 hours after sonication for histological analysis and DOX quantification, respectively.

Figure 2.

MRI evaluation of FUS-induced BBB disruption and tissue damage in the rat brain. A. Time series of axial contrast-enhanced FSE T1-weighted images showing localized BBB disruption at two sonicated spots (arrows). The images show contrast changes relative to pre-contrast images. B. Sagittal and coronal views. C. T2*-weighted images in axial and coronal planes showed no evidence of extensive petechiae, which would appear in this imaging as hypointense spots. The PRFP amplitude was 0.72 and 0.68 MPa in locations 1 and 2, respectively. Scale bars are 5 mm.

Figure 3.

Example MRI and permeability estimates for the cases that had the highest (A–D) and the lowest (right: E–H) BBB permeability changes after a single sonication. (A, E) Axial contrast-enhanced T1-weighted images and (B, F) corresponding permeability maps obtained after Gd-DTPA injections at four time-points. K_{trans} values (units: min^{-1}) are indicated in the color bar. (C, G) Mean K_{trans} as a function of time for $0.75 \times 0.75 \text{ mm}^2$ regions of interest at the sonicated targets and for corresponding non-sonicated locations. For the controls, regions of interest were selected in the contralateral hemisphere at the same anatomical locations that were sonicated. In each case an elevated K_{trans} was observed after sonication, which decayed over time. (D, H) Coronal T2*-weighted imaging showed no evidence of hypointense spots that would indicate that extensive erythrocyte extravasation was produced during these sonications. The PRFP amplitude was 0.72 and 0.68 MPa at locations 1 and 2, respectively. Scale bars are 5 mm.

Figure 4.

Mean K_{trans} values measured from DCE-MRI in regions of interest at the targeted locations and in corresponding non-sonicated structures in the contralateral hemisphere. The decay of K_{trans} , which occurred due to restoration of the BBB, was fit to an exponential decay (solid line; dotted lines: 95% CI). (A) After a single sonication, the mean K_{trans} at the sonicated targets was significantly larger than that measured in the control locations (** $P < 0.001$ and * $P < 0.05$). Data shown are mean K_{trans} values ($\pm$ S.D) measured at 11 sonicated spots in six rats. (B–C) Double sonications were applied at the same targeted spot with a 10 (B) or 120 (C) min time interval between sonications. Data shown are mean K_{trans} values ($\pm$ SD) measured at six sonicated locations in three rats for (B) and at five locations in three rats for (C). The mean K_{trans} at the sonicated targets was significantly larger than that measured in the control locations at all time points except one (** $P < 0.001$ and * $P < 0.05$).

Figure 5.

DOX concentration achieved at the sonicated locations as function of K_{trans} measured using DCE-MRI 30 min after sonication. The DOX concentration was measured approximately 16 hours later for brain targets in single sonication (SS), single sonication with hyperintense spots in T2* weighted image (SS (+T2*)), double sonication with 10 min interval (DS 10min), and double sonication with 120 min interval (DS 120min). The solid line shows a linear regression of the data (slope: 28,824 ng/g DOX per change in K_{trans} in min⁻¹; intercept: 377 ng/g DOX; R: 0.7). For the animals sonicated a second time after a 120 min interval, the K_{trans} measurements obtained after the first sonication are shown, as the second sonication was delivered after DOX had cleared from the bloodstream.

Figure 6.

DOX concentrations measured approximately 16 hours after FUS in the sonicated brain regions (gray bars) and at control locations in the contralateral hemisphere (white bars) for the different experimental groups. For all groups, the sonicated locations had a significantly higher DOX concentration than the control targets ($p < 0.0001$). Targets sonicated twice with a 10 min delay between the exposures had a significantly higher DOX concentration than the other groups ($p < 0.05$). This group achieved a concentration that exceeded a concentration shown by others that could be correlated with a clinical response in breast cancer patients (819 ± 482 ng/g tumor).

Figure 7.

Microphotographs of H&E stained sections for three rats that received a SS (B and F), DS with a 120 min interval between sonications (C and G) and DS with 10 min interval (A, D, E, and H). (A–D) were obtained with a 10 $\times$ objective. (E–H) are magnified views (40 $\times$) of the boxes. Most of the sonicated region appeared unaffected (A and E); these examples (B–D and F–H) show the most severe petechiae that were found in these animals. In each case, MRI contrast enhancement was confirmed after sonication, and no hypointense regions, indicative of severe red blood cell extravasation, were evident in T2*-weighted imaging. These animals were sacrificed four hours after the last sonication. Scale bars are 100 μ m.

Table 1

Summary of the experimental groups

Group number	Experiments	No. of rats	No. of sonication/BBBD	Pressures amplitude (MPa)
1	BBBD/DCE Parameters and Histology	8	16	0.6 – 0.8
2	Single Sonication	6	12/12 ^a	0.68 and 0.72
3	Double Sonication 10 min Interval	3	6/6	0.68 and 0.72
4	Double Sonication 2 hr Interval	3	6/5 ^b	0.68 and 0.72

^aBrain damage was detected in T2* weighted images in one spot.^bBBBD was not detected in DCE or T1-weighted FSE imaging after sonication in one target location.

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Table 2

The MRI parameters used in the study

Sequence	Purpose	FOV (mm)	RT (ms)	ET (ms)	Matrix	FA (°)	SL (mm)	ST (s)	BW (±kHz)
FSE T2-W	Tissue anatomy targeting	80	2000	85	256 ×256	90	1	132.2	16
FSPGR	T1 mapping	80	9.9	4.8	256 ×256	30/20/1 5/10/2	2	15.3 [§]	31.25
FSPGR	DCE	80	9.9	4.8	256 ×256	35	2	534.6	31.25
FSE T1-W	Contrast enhancement	80	500	17	256 ×256	90	1	130.2	16
SPGR T2*-W	Tissue damage	80	33	19	256 ×256	15	0.7	276.7	16

FOV: field of view, RT: Repetition time, ET: Echo time, FA: Flip angle, SL: Slice thickness, ST: Scan time, BW: Bandwidth

[§]Scan time for each flip angle

Table 3

Parameters estimated for exponential decay of K_{trans} (equation 5) for the three experimental groups

Group	R (hrs)		Half-life (hrs)		K_{trans}^0 (min^{-1})		R^a
	Mean	CI	Mean	CI	Mean	CI	
SS	3.20	(1.52–4.89)	2.22	(1.06–3.39)	0.0163	(0.012–0.021)	0.98
DS (10 min)	2.60	(1.73–3.47)	1.80	(1.20–2.41)	0.0205	(0.016–0.025)	0.97
DS (120 min)	4.82	(1.22–8.43)	3.34	(0.84–5.84)	0.0216	(0.013–0.030)	0.99

^a correlation coefficient of nonlinear regression