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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 μl/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 hrs after sonication using DCE-MRI. After a single sonication (SS), the mean K_{trans} was 0.0142 ± 0.006 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 hrs (95% confidence intervals (CI): 1.06–3.39 hrs). 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 hrs (CI: 1.20–2.41 hrs) for a 10 min interval between sonications and increased to 3.34 hrs (CI: 0.84–5.84 hrs) 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.

1. 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 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

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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 4 hrs 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 hrs after FUS-induced BBBD [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 BBBD. 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 BBBD and the time at which the BBB is disrupted.

2. Materials and methods

2.1. 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.

2.2. 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 3 T MRI scanner (General Electric Healthcare, Milwaukee, WI). MRI was used for image guidance and BBBD 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.

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

[a] Brain damage was detected in T2* weighted images in one spot.
[b] BBBD was not detected in DCE or T1-weighted FSE imaging after sonication in one target location.

2.3. Sonications

Burst sonications (10 ms bursts applied at 1 Hz for 60 s) at acoustic power levels ranging from 0.34 to 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 C_3F_8 gas encapsulated by an outer phospholipid shell. Immediately after