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Targeted drug delivery with focused ultrasound-induced blood-brain barrier opening using acoustically-activated nanodroplets

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ABSTRACT

Focused ultrasound (FUS) in the presence of systemically administered microbubbles has been shown to locally, transiently and reversibly increase the permeability of the blood–brain barrier (BBB), thus allowing targeted delivery of therapeutic agents in the brain for the treatment of central nervous system diseases. Currently, microbubbles are the only agents that have been used to facilitate the FUS-induced BBB opening. However, they are constrained within the intravascular space due to their micron-size diameters, limiting the delivery effect at or near the microvessels. In the present study, acoustically-activated nanodroplets were used as a new class of contrast agents to mediate FUS-induced BBB opening in order to study the feasibility of utilizing these nanoscale phase-shift particles for targeted drug delivery in the brain. Significant dextran delivery was achieved in the mouse hippocampus using nanodroplets at clinically relevant pressures. Passive cavitation detection was used in the attempt to establish a correlation between the amount of dextran delivered in the brain and the acoustic emission recorded during sonication. Conventional microbubbles with the same lipid shell composition and perfluorobutane core as the nanodroplets were also used to compare the efficiency of an FUS-induced dextran delivery. It was found that nanodroplets had a higher BBB opening pressure threshold but a lower stable cavitation threshold than microbubbles, suggesting that contrast agent-dependent acoustic emission monitoring was needed. A more homogeneous dextran delivery within the targeted hippocampus was achieved using nanodroplets without inducing inertial cavitation or compromising safety. Our results offered a new means of developing the FUS-induced BBB opening technology for potential extravascular targeted drug delivery in the brain, extending the potential drug delivery region beyond the cerebral vasculature.

1. Introduction

Focused ultrasound (FUS)-induced blood–brain barrier (BBB) opening has been shown to hold great promise for targeted drug delivery in the brain for the treatment of central nervous system diseases, including Alzheimer's [1], Huntington's [2] and Parkinson's [3] diseases as well as brain cancers [4,5]. Various therapeutic agents, including antibodies [6], neural stem cells [7], siRNA [2], chemotherapeutic molecules [8] and neurotrophic factors [3], have been delivered across the BBB and shown capable of inducing therapeutic effects using this technology. The FUS-microbubble-based drug delivery system relies on the capability of systemically-administered and acoustically-activated microbubbles to non-invasively, transiently and reversibly increase the cellular and vascular permeability of the BBB, thus allowing drug molecules that are normally restricted within the vasculature to cross the barrier and be delivered to the brain parenchyma. Previous studies by our group and others have shown that the extent of the BBB opening, as characterized by the opening volume, opening size and opening duration based on magnetic resonance or fluorescence imaging, is directly linked to the acoustic energy applied during sonication [9–12] and the physicochemical properties of the microbubbles used [6,13,14]. Informed selections of the acoustic exposure parameters and the type of contrast agent are critical for an effective drug delivery while minimizing the possibility of side effects. Not accounting for these factors may result in unintended adverse bioeffects [15] or even undesired brain damage in more severe situations [9].

Real-time passive acoustic emissions from the oscillating microbubbles could offer means to identify signature characteristics that allow for the differentiation of the complex behaviors of microbubble cavitation during sonication [16–19]. During stable cavitation, the magnitude of microbubble oscillation depends on the acoustic pressure applied. Such behavior has been assumed to induce sufficient forces on the endothelium through either radiation or shear forces induced by microstreaming around the microbubbles [19]. Shear stress-induced endocytosis could also be a

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potential mechanism to increase the transcellular permeability [20]. As the acoustic energy is increased, a larger radial microbubble expansion could induce inertial cavitation, leading to microbubble collapse and potentially generating liquid microjets to form pores within the cell membrane improving the cellular update of nanoparticles and drugs [21]. The disruption of the tight junction proteins between the adjacent brain endothelial cells during inertial cavitation could also increase the paracellular BBB permeability [22]. Regardless of the exact mechanism, the cavitation emission during sonication could be calculated and correlated with the outcome of the BBB opening, thus suggesting that the acoustic signature of the microbubbles could serve as a predictive indicator for estimating the amount of drug delivered to the targeted region [16,23].

Currently, FUS-induced BBB opening has only been achieved using commercially available [1,14,24] or in-house manufactured [25,26] gaseous microbubbles. Despite their utility as mediating vehicles, microbubbles can only be used as vascular contrast agents since their size (typically 1–10 μm) prevents their extravasation, limiting the cavitation effects to regions at or near the vessels. Using fluorescence microscopy, our group [27] previously showed that homogeneous distribution of relatively larger dextran molecules (10 kDa and 70 kDa) was more difficult to achieve than with smaller ones (3 kDa), highlighting the need for an agent with greater ability to induce molecular diffusion beyond the cerebral vasculature. In addition, gaseous microbubbles have short *in vivo* circulation half-life, typically on the order of minutes, thus requiring re-administration if repeated sonication is desired.

Phase-shift perfluorocarbon droplets may potentially offer the solution to address these issues [28,29]. Several groups have demonstrated the therapeutic utility of this alternative class of ultrasound contrast agents for applications such as targeted vessel occlusion [30], ablation enhancement [31], and drug delivery to solid tumors [32,33]. Initially in the liquid state, nanoscale droplets show high stability in circulation (i.e., on the order of hours) and can be generated in sizes small enough to extravasate through leaky vasculature; but once exposed to sufficient rarefactional pressures they expand to form microbubbles capable of oscillation in a similar fashion as traditional microbubbles—ideal for ultrasound-mediated imaging and therapy. For the purposes of BBB opening, these agents may present a unique opportunity. Because droplet activation is dependent on the local rarefactional pressure, the microbubbles will only be generated within the narrow focal region where acoustic properties are the strongest, ensuring that the therapeutic effect is limited to the desired treatment areas [34,35]. Once initial BBB permeabilization is achieved, the droplets remaining will be small enough for potential extravasation during subsequent passes through the vasculature. Once entered into the interstitial space behind the barrier, the droplets could then be acoustically activated to form vaporized gaseous bubbles outside the constraints of the cerebral microvessels. Therefore, the possibility exists for an extravascularly activated contrast agent for enhanced drug delivery at sites that are located deeply in the brain tissue or at regions with relatively low vasculature density. Activation of typical nanodroplet formulations requires acoustic pressures which are much higher than those used for FUS-induced BBB opening [36,37], but recent studies have shown that it is possible to generate nanodroplets from highly volatile perfluorocarbons by pressurizing preformed microbubbles and condensing the gas core into liquid phase during slow cooling [23,38]. This methodology can produce uniform nanodroplet size distributions with peak diameters near 200–300 nm that vaporize at acoustic pressures on the order of those required for BBB opening with microbubbles.

The main objective of the present study was to show the feasibility of utilizing phase-shift nanodroplets as the mediating agents for targeted drug delivery using FUS-induced BBB opening *in vivo*. Conventional in-house generated microbubbles with the same lipid shell composition and perfluorocarbon gaseous core were also used as the "standard" for comparing the efficiency of BBB opening based on targeted delivery of fluorescent dextran molecules. A series of clinically relevant acoustic exposure conditions were used to further demonstrate the safety of this technology for drug delivery in the brain.

2. Materials and methods

2.1. Contrast agent generation

Nanodroplets and microbubbles were formulated using the same lipid composition containing 90 mol% 1,2-distearoyl-sn-glycero-3-phosphocholine (DSPC) and 10 mol% 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)2000] (DSPE-PEG2000) (Avanti Polar Lipids, Alabaster, AL). The perfluorobutane gas (PFB, 99 wt.% purity) used for contrast agent generation was obtained from FluoroMed (Round Rock, TX). All chemicals were used as purchased without further purification.

Microbubbles were generated via mechanical agitation using a Vialmix shaker (Bristol–Myers–Squibb, New York, NY). A Multisizer III particle counter (Beckman Coulter, Opa Locka, FL) with a 30 μm aperture was used to measure the microbubble suspension size distribution and concentration. Nanodroplets were generated via microbubble condensation, in which pre-formed microbubbles of volatile compounds were reverted to the liquid state by application of reduced temperature and increased ambient pressure [23,38]. Briefly, PFB microbubbles were generated as described above and allowed to cool to room temperature. The vial containing the microbubbles was then immersed in a CO_2/isopropanol bath maintained at a temperature between −7 °C and −10 °C for approximately 1 min. The vial was subsequently connected to an adjustable air-pressure supply, and the headspace pressure inside the microbubble vial was increased by 30–70 kPa for approximately 30 s to facilitate condensation. A Malvern Nano Zetasizer (Malvern Instruments Ltd., Malvern, Worcestershire, U.K.) was used to measure the size distribution of the droplet emulsion generated.

2.2. In vitro acoustic nanodroplet vaporization setup

Acoustic nanodroplet vaporization was investigated *in vitro* using an experimental setup described previously [39]. An ultra-high-speed framing camera with a 24-frame buffer (SIMD24; Specialised Imaging, Simi Valley, CA) was interfaced with an inverted microscope (IX71; Olympus, Center Valley, PA) with a 100× (NA = 1.0) water immersion objective. An acrylic-lined, continuously degassed water bath was mounted to the microscope and maintained at 37 °C. The optical resolution of the system allowed observation of particles larger than approximately 500 nm. A 1 MHz spherically-focused piston transducer with a 2.2 cm diameter and a focal length of 3.75 cm (IL0106HP; Valpey Fisher Corp., Hopkinton, MA) was aligned with the optical focus by the use of a calibrated needle hydrophone (HNA-0400; Onda Corp., Sunnyvale, CA). The transducer was driven by sinusoidal 20-cycle pulses generated by a manually-triggered arbitrary waveform generator (AFG 3101; Tektronix, Inc., Beaverton, OR) amplified 60 dB (A500; ENI, Rochester, NY). The sinusoid amplitude was adjusted to change the peak rarefactional pressure experienced by the droplets in focus. The manual trigger was synchronized with the input of the high-speed camera in order to simultaneously capture a video of droplet vaporization. Droplet emulsions were diluted 50% in phosphate-buffered saline (PBS) and pumped through a nearly optically and acoustically transparent microcellulose tube (Spectrum Laboratories, Inc., Greensboro, NC). The focal plane of the tube was controlled via a 3-axis micropositioner (MMO-203; Narishige Group, East Meadow, NY). The ultra-high-speed camera was set to operate at