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Multiple sessions of liposomal doxorubicin delivery via focused ultrasound mediated blood–brain barrier disruption: A safety study

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ABSTRACT

Transcranial MRI-guided focused ultrasound is a rapidly advancing method for delivering therapeutic and imaging agents to the brain. It has the ability to facilitate the passage of therapeutics from the vasculature to the brain parenchyma, which is normally protected by the blood–brain barrier (BBB). The method's main advantages are that it is both targeted and noninvasive, and that it can be easily repeated. Studies have shown that liposomal doxorubicin (Lipo-DOX), a chemotherapy agent with promise for tumors in the central nervous system, can be delivered into the brain across BBB. However, prior studies have suggested that doxorubicin can be significantly neurotoxic, even at small concentrations. Here, we studied whether multiple sessions of Lipo-DOX administered after FUS-induced BBB disruption (FUS-BBBD) induces severe adverse events in the normal brain tissues. First, we used fluorometry to measure the doxorubicin concentrations in the brain after FUS-BBBD to ensure that a clinically relevant doxorubicin concentration was achieved in the brain. Next, we performed three weekly sessions with FUS-BBBD ± Lipo-DOX administration. Five to twelve targets were sonicated each week, following a schedule described previously in a survival study in glioma-bearing rats (Aryal et al., 2013). Five rats received three weekly sessions where i.v. injected Lipo-DOX was combined with FUS-BBBD; an additional four rats received FUS-BBBD only. Animals were euthanized 70 days from the first session and brains were examined in histology. We found that clinically-relevant concentrations of doxorubicin (4.8 ± 0.5 μg/g) were delivered to the brain with the sonication parameters (0.69 MHz; 0.55–0.81 MPa; 10 ms bursts; 1 Hz PRF; 60 s duration), microbubble concentration (Definity, 10 μl/kg), and the administered Lipo-DOX dose (5.67 mg/kg) used. The resulting concentration of Lipo-DOX was reduced by 32% when it was injected 10 min after the last sonication compared to cases where the agent was delivered before sonication. In histology, the severe neurotoxicity observed in some previous studies with doxorubicin by other investigators was not observed here. However, four of the five rats who received FUS-BBBD and Lipo-DOX had regions (dimensions: 0.5–2 mm) at the focal targets with evidence of minor prior damage, either a small scar (n = 4) or a small cyst (n = 1). The focal targets were unaffected in rats who received FUS-BBBD alone. The result indicates that while delivery of Lipo-DOX to the rat brain might result in minor damage, the severe neurotoxicity seen in earlier works does not appear to occur with delivery via FUS-BBB disruption. The damage may be related to capillary damage produced by inertial cavitation, which might have resulted in excessive doxorubicin concentrations in some areas.

1. Introduction

The blood–brain barrier (BBB) is one of the most challenging factors for effective diagnosis and treatment of brain diseases. It prevents the extravasation of most circulating therapeutics and imaging agents into the brain because of its selective permeability to only a small subset of molecules that have the correct size, charge and lipid solubility [1,2]. Invasive approaches such as direct injection, infusion, and implanted biocompatible devices have been used to achieve local high drug concentration [3–6]. Others have had promising results with biopharmaceutical approaches such as the modification of drugs to cross the barrier through endogenous transport mechanisms [7–9]. However, all current methods are either invasive, non-targeted, or require the expense of developing new drugs. A drug targeting technology that could noninvasively achieve controlled delivery of therapeutics across the BBB would be highly beneficial.

Over a decade ago, Hynynen et al. [10] discovered that the BBB can be temporarily disrupted with low-intensity bursts of focused ultrasound combined with circulating microbubbles. This method has several potential advantages over other approaches tested to overcome the BBB [11]. It is a noninvasive procedure, and effect can be localized to only desired volumes in the brain. Since that work was published, the method has been investigated in numerous animal studies as a noninvasive targeted drug delivery method [12]. These studies have demonstrated the delivery of a wide range of imaging and therapeutic agents

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including large agents such as antibodies, nanoparticles, and liposomally-encapsulated drugs [13–16]. They have also demonstrated that the BBB can be consistently disrupted without apparent neuronal damage [10,17–22]. The circulating microbubbles appear to concentrate the ultrasound effects to the blood vessel walls, causing BBB disruption through widening of tight junctions and activation of transcellular mechanisms, with little apparent effect on the surrounding parenchyma [23]. The use of injected microbubbles also makes the method more predictable than prior studies that used ultrasound alone [24–26] and reduces the acoustic power needed for BBB disruption by orders of magnitude, making FUS-BBBD substantially easier to apply through the intact skull without overheating the bone.

One area that will likely benefit the most from transient BBB disruption is the use of chemotherapy for the treatment of brain tumors. BBB disruption in conjunction with chemotherapy has been investigated intensively for several decades using intra-arterial injection of hyperosmotic solutions such as mannitol. This procedure causes shrinkage of endothelial cells and consequent stretching of tight junctions [27] through which drugs may pass. The method has been tested clinically with promising results [28–33]. The use of focused ultrasound to disrupt the BBB has the potential to replicate these findings without requiring an invasive procedure and at the same time targeting the chemotherapy delivery to only desired regions.

The chemotherapy agent doxorubicin (molecular weight: 580 Da) has been shown to be effective against glioma cells in vitro [34], but not in patients [35]. The poor clinical outcomes were presumably the result of the BBB and other challenges inherent in tumor drug delivery [11,36]. This agent is often used in a liposomal formulation, which reduces cardiotoxicity and other side effects but also makes drug delivery even more challenging due to the large size (~100 nm) after encapsulation. Several studies have shown that FUS-BBBD can enable the delivery of doxorubicin, either alone or encapsulated in a liposome, across the BBB and enhance its delivery across "blood–tumor barrier" [37–40]. Other works have demonstrated improvements in survival and decreased tumor growth in animal tumor models [38,41]. Recently, we investigated three weekly sessions of FUS-BBBD to enhance the delivery of liposomal doxorubicin (Lipo-DOX) to a rat glioma model and to enable its delivery across the BBB in the surrounding brain [42]. A pronounced improvement was observed: the median survival time was increased by 100% and 72% compared to controls and animals who received only Lipo-DOX, respectively; approximately 75% of the tumors appeared almost completely resolved. However, some adverse events were observed, including tissue loss at the tumor site, damage (infarct) in neighboring tissue, and intratumoral hemorrhage in one animal. We could not determine whether these effects were due to the sonications, the chemotherapy, or the tumors themselves, which in some cases reached a substantial volume before beginning to resolve.

In considering clinical translation, it will be critical to understand if these side effects were due to doxorubicin neurotoxicity. An effective drug treatment for an invasive brain tumor such as glioma will require chemotherapy delivery to the normal tissues at the tumor margin, where the BBB protects infiltrating tumor cells, in addition to the semi-permeable solid tumor. In a patient with a glioma, this infiltrative margin can extend several centimeters [43]. While an earlier study of FUS-BBBD and Lipo-DOX found that a single drug delivery session did not result in the normal brain tissue damage in rats [44], it is possible that multiple treatments could result in the side effects observed in our prior tumor study [42]. Furthermore, early studies with mannitol BBB disruption and free doxorubicin suggested that this drug is significantly neurotoxic, even at small concentrations [45,46]. Others have observed concentration-dependent neurotoxicity when free doxorubicin or Lipo-DOX was infused into the brain via convection-enhanced delivery [47].

For these reasons, we tested whether multiple sessions of Lipo-DOX administration and FUS-mediated BBB disruption (FUS-BBBD) can induce severe adverse events in the normal brain tissue. The present study had two objectives. First, we aimed to confirm that the sonication parameters used in our prior study with FUS-BBBD and Lipo-DOX [42] can deliver clinically-relevant concentrations of doxorubicin and to test whether injecting the agent before, during, or after the sonications influences the resulting drug concentrations. Next, sonicating multiple targets in the normal brain over three weeks, we evaluated whether multiple sessions of FUS-BBBD and Lipo-DOX produced significant brain tissue damage. For these experiments, we aimed to recreate the sonications used in our earlier tumor study. We sonicated multiple overlapping brain targets to induce BBB disruption in regions that increased in volume over the three weeks. The tissue effects were compared in histology to animals who received FUS-BBBD or Lipo-DOX alone.

2. Materials and methods

2.1. Sonication system

An air-backed, single element, 690 kHz focused piezoelectric transducer (diameter/radius of curvature: 100/80 mm) generated the ultrasound field. It was driven by an arbitrary waveform generator (model 395, Wavetek) and RF amplifier (240 L, ENI); electric power was measured with a power meter (E4419B, Agilent,) and dual-directional coupler (C5948-10, Werlatone). Reported exposure levels are absolute peak negative pressure amplitudes measured in water with a membrane hydrophone (Marconi; 0.5 mm diameter). Attenuation by the brain and rat skull is expected to reduce the pressure amplitude by ~30% at this frequency [48] with additional uncertainty arising from standing waves within the skull and increases in skull thickness as the animal ages [48]. The pressure distribution of the transducer was mapped using a 0.2 mm needle hydrophone (Onda, Sunnyvale, CA); its half-maximum diameter and length were 2.3 and 12 mm, respectively. The transducer efficiency was measured using a radiation force-balance.

Acoustic parameters were the same as in our previous study [42]. The sonications consisted of 10 ms bursts applied at a frequency 1 Hz for 60 s at a pressure amplitude of 0.55 MPa. This pressure amplitude was initially set based on a prior study in rats with this device [17] and was increased on the basis of the animal age and weight to achieve a consistent level of BBB disruption. This observation was made in our initial treatments and is similar to previous reports [48]. Each sonication was combined with an intravenous injection of a microbubble-based ultrasound contrast agent (Definity; Lantheus) administered at the dose recommended for human ultrasound imaging (10 μl/kg). Each milliliter of Definity contains 1.2×10^{10} microbubbles that consist of perfluorocarbon gas-filled lipid shells with a mean diameter of 1.1–3.3 μm. To facilitate the injections of such a small volume, the agent was diluted in PBS to 0.1 times its normal concentration. It was injected as a bolus approximately 9 s before each sonication, followed by a 0.2 ml saline flush.

2.2. Experimental setup

The sonication system was operated within a clinical 3T MRI scanner (Signa; GE Healthcare). The transducer was immersed in a small tank of degassed, deionized water and attached to an MRI-compatible, manually-operated positioning system (Fig. 1). The animal was laid supine on a tray above this tank, with a water bag providing an acoustic path to the dorsal surface of the head. Images were obtained with a 7.5 cm-diameter transmit/receive MRI surface coil. The animal's body temperature was maintained with a heated water pad. Before the rat experiments, we visualized heating in a silicone phantom using temperature-sensitive MRI to localize the acoustic focal point in the MRI space. Accurate targeting in vivo was confirmed before the sonications in select animals (typically the first animal sonicated each week) by verifying that the resulting MRI contrast extravasation appeared at the desired target after one sonication.