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Evaluation of permeability, doxorubicin delivery, and drug retention in a rat brain tumor model after ultrasound-induced blood-tumor barrier disruption

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ABSTRACT

Drug delivery in brain tumors is challenging because of the presence of blood-brain barrier (BBB) and the blood-tumor barrier (BTB). Focused ultrasound (FUS) combined with microbubbles can enhance the permeability of the BTB in brain tumors, as well as disrupting the BBB in the surrounding tissue. In this study, dynamic contrast-enhanced Magnetic Resonance Imaging (DCE-MRI) was used to characterize FUS-induced permeability changes in a rat glioma model and in the normal brain and to investigate the relationship between these changes and the resulting concentration of the chemotherapy agent doxorubicin (DOX). 9L gliosarcoma cells were implanted in both hemispheres in male rats. At day 10–12 after implantation, FUS-induced BTB disruption using 690 kHz ultrasound and Definity microbubbles was performed in one of the tumors and in a normal brain region in each animal. After FUS, DOX was administered at a dose of 5.67 mg/kg. The resulting DOX concentration was measured via fluorometry at 1 or 24 h after FUS. The transfer coefficient K_{trans} describing extravasation of the MRI contrast agent Gd-DTPA was significantly increased in both the sonicated tumors and in the normal brain tissue ($P < 0.001$) between the two DCE-MRI acquisitions obtained before and after FUS, while no significant difference was found in the controls (non-sonicated tumor/normal brain tissue). DOX concentrations were also significantly larger than controls in both the sonicated tumors and in the normal tissue volumes at 1 and 24 h after sonication. The DOX concentrations were significantly larger ($P < 0.01$) in the control tumors harvested 1 h after FUS than in those harvested at 24 h, when the tumor concentrations were not significantly different than in the non-sonicated normal brain. In contrast, there was no significant difference in the DOX concentrations between the tumors harvested at 1 and 24 h after FUS or in the concentrations measured in the brain at these time points. The transfer coefficient K_{trans} for Gd-DTPA and the drug concentrations showed a good linear correlation ($R^2 = 0.56$). Overall, these data suggest that FUS and microbubbles can not only increase DOX delivery across the BBB and BTB, but that it is retained in the tissue at significantly enhanced levels for at least 24 h. Such enhanced retention may increase the potency of this chemotherapy agent and allow for reduced systemic doses. Furthermore, MRI-based estimates of Gd-DTPA transport across these barriers might be useful to estimate local DOX concentrations in the tumor and in the surrounding normal tissue.

1. Introduction

Treatment of brain tumors remains a great challenge because of the presence of the blood-brain barrier (BBB) and the partially-intact blood-tumor barrier (BTB). These barriers preclude the effective passing of most chemotherapeutics from the blood circulation to the brain parenchyma and limit their delivery to tumors [1]. Different methods have been used to overcome those barriers and have had promising outcomes, but they all have been either invasive, non-targeted, or required the formulation of new drugs [2]. Focused ultrasound (FUS) has emerged with a great promise to temporarily permeabilize these vascular barriers and enable or enhance drug delivery for brain tumors and other disorders of the central nervous system [3]. When burst ultrasound is combined with microbubbles, mechanical effects produced by the acoustically activated microbubbles are localized to the vasculature and result in temporary opening of the BBB and BTB. This method is accessible transcranially without the need of invasive procedures because it can be achieved using low frequency ultrasound (<1 MHz) that can penetrate the intact skull [4]. Several studies have been shown that FUS-induced BBB opening is safe, even with multiple sessions [5–9].

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With the use of this technique, a wide range of imaging and therapeutic agents have been delivered into the brain [10–13] as well in tumor models in animals [14–17].

Given that the vascular density and permeability can vary substantially in tumors, it could be useful to have a method to predict and map how much drug is delivered to the targeted tissue volume. This can be achieved directly by radiolabeling drugs or by conjugating them to imaging contrast agents. It may also be possible to estimate drug delivery using a surrogate marker. Dynamic contrast-enhanced MRI (DCE-MRI) has been performed to estimate the spatial and temporal characteristics of BBB permeability after FUS [18,19]. The relationship between the delivery of MRI contrast agents and the resulting concentration of drugs or other tracers has been explored in normal brain and in murine tumor models [20–24]. These studies suggest that MRI contrast agents can predict drug or tracer concentrations in the normal brain, even when large drug carriers (liposomes) are used [11, 22,25,26]. In a tumor model, others have found a good correlation between MRI contrast and Evan's Blue delivery a rat tumor model [21, 23]. However, we did not find such an agreement with between MRI contrast and liposomal doxorubicin delivery in tumors [24].

Along with concentration, the amount of time that a drug resides in the tissue after it extravasates may have an impact on its therapeutic efficacy. Clearance of drugs from the brain occurs through the "glymphatic" system [27]. This system, along with the transport of many substances back into the blood stream via drug efflux pumps such as P-glycoprotein [28,29], and other factors [30,31], result in a highly regulated chemical environment. These clearance mechanisms differ from what occurs in most other tissues and might affect how long different drugs are present in the brain or a brain tumor. It may also possible that ultrasound-induced effects could enhance this duration, through enabling greater drug penetration or by modulating drug efflux. If drug retention could be increased, it might allow for the use of a lower systemic drug dose.

The purpose of this work was to investigate drug concentrations at different time points in healthy brain tissue and in a brain tumor model after ultrasound-induced BBB/BTB permeabilization. We used free (unencapsulated) doxorubicin (DOX), a chemotherapy agent with a relatively low molecular weight (580 Da) that is cleared rapidly from the body after administration as a model drug. DOX concentrations were correlated with estimates of permeability changes for an MRI contrast agent made using DCE-MRI.

2. Materials and methods

2.1. Animals

The experiments were approved by our institutional animal care and use committee. A total of 21 male Sprague-Dawley rats (Charles River Laboratories, Boston, MA; weight: 250–280 g) were used for this study. The animals were randomly divided into five groups (Table 1). Before each procedure, the rats 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 i.p. injection. A catheter was placed in the tail vein for i.v. injection of microbubbles, MRI contrast agent, DOX, or Trypan blue.

Table 1
Summary of the experimental groups.

Group number	Experimental purpose	No. of rats	Sonication (right/left)	Pressure amplitude (MPa)
1	Normal brain	5	5/5	0.72
2	Brain tumor-1 h	6	6/0	0.72
3	Brain tumor-24 h	5	5/0	0.72
4	Histology	2	2/0	0.72
5	Control/baseline signal		n/a	n/a

2.2. 9L cell implantation for brain tumor.

The 9L rat gliosarcoma cell line was provided by the Neurosurgery Tissue Bank at The University of California-San Francisco. The cells were grown in Minimum Essential Medium (MEM) with Earle's salts, supplemented with 10% heat-inactivated fetal bovine serum, 1% L-glutamine, 1% MEM nonessential amino acids, and 0.1% gentamicin (Invitrogen, Carlsbad, CA) and maintained at 37 °C in a humidified incubator containing 5% CO_2. Rats were anesthetized, the hair on the scalp was removed with clippers and depilatory cream (Nair, Church & Dwight Co., Inc., Princeton, NJ), and the dorsal surface of the skull was sterilized with a povidone-iodine swab and alcohol. The head was immobilized in a stereotactic frame. After a skin incision to expose the skull, a 1 mm burr hole was drilled into the skull 2 mm lateral and 1 mm anterior of the bregma. Immediately before implantation, the 9L cells were trypsinized and resuspended in serum-free MEM, containing no additional supplements. A 10-μl Hamilton syringe (Hamilton, Reno, Nevada) was inserted 3.5 mm into the cerebral cortex. A 4 μl volume of cell suspension (1×10^5 cells in MEM) was injected over a 5 min period. After waiting 2 min, the needle was retracted slowly over another 5 min. The hole in the skull was sealed with bone wax (Ethicon, Somerville, New Jersey) to prevent leakage of the cerebrospinal fluid, and the incision was closed with 5–0 silk sutures (Ethicon, Somerville, New Jersey). Each animal was given a one-time dose of antibiotic (Baytril, 2.5 mg/kg; Bayer Healthcare, Wayne, New Jersey) and an analgesic (Buprenex, 0.05 mg/kg; Reckitt Benckiser Healthcare, Hull, England, UK) every 12 h for 24 h following the implantation by i.p. administration. The sutures were removed prior to sonication one week after tumor implantation.

2.3. Sonications

An air-backed, single-element, spherically-curved, piezoelectric transducer with a diameter of 100 mm, a radius of curvature of 80 mm, and a resonant frequency of 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 in water. The half-maximum pressure amplitude width and length of the focal region for this transducer were 2.3 and 14 mm, respectively. 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. The transducer was immersed in a tank of degassed water and mounted on an MR-compatible positioning system (Fig. 1A). The experiments were performed in a clinical 3 T MRI scanner (General Electric Healthcare, Milwaukee, WI). MRI was used for image guidance and evaluation of BBBD/BTBD. The imaging was performed using a 7.5 cm diameter transmit/receive surface coil (constructed in-house).

Five targets in and around the tumor in right hemisphere were exposed to ultrasound (10 ms bursts period with at 1 Hz pulse repetition frequency for 60 s) transcranially into the brain of the rat, which laid in the supine position on the sonication system (Fig. 1A). The ultrasound power amplitude was 0.68–0.72 MPa. These exposure levels used were obtained from a prior safety study with this transducer in rats [7]. Similar locations in the tumor were selected in the left hemisphere. For the normal rats, similarly, five targets at each hemisphere were