## Natalia Vykhodtseva, Yuexi Huang and Kullervo Hynynen HISTOLOGICAL FINDINGS IN THE BRAIN AFTER FOCUSED ULTRASOUND ABLATION COMBINED WITH DEFINITY USING PARAMETERS SUITED FOR TRANSCRANIAL APPLICATION

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Previous experiments with ultrasound contrast (US) agent, Optison and 1.5MHz focused ultrasound (FUS) demonstrated a dramatic reduction in power needed to produce well-defined lesions in the brain. In this exploratory study, we investigated such ablation with US contrast agent, Definity and exposures more suited for transcranial FUS. Burst sonications (10ms, PRF: 1Hz; peak pressure amplitude: 0.35-0.58 MPa) were applied transcranially in the brains of rats (n=12) using a 558 kHz transducer (diameter/ROC: 10/7.8 cm). Histological changes at 24h, 48h, and 7d were characterized in light microscopy. Discrete lesions were created at substantially lowered acoustic power levels when FUS was combined with Definity. In histology, the lesions exhibited red blood cell extravasations and destruction of blood vessels, presumably caused by inertial cavitation. The cells then died mainly due to ischemia. These histological findings suggest that FUS combined with the Definity produce localized lesions mostly through cavitation-induced damage to blood vessels. The edges of the lesions were not well-defined and it was difficult to determine the full lesion extent. Several mm away from the apparent edge the lesions, additional hemorrhages were sometimes observed, especially at interfaces between different brain structures and in the ventricles. This work demonstrates that ultrasound contrast agent can be combined with 558 kHz ultrasound to produce lesions in the brain. Future work will be necessary to optimize the exposures and create more well-defined lesions.

# INTRODUCTION

In recent years, high-intensity focused ultrasound (HIFU) has emerged as a non-invasive method for thermal ablation of a variety of tumors, including those of the uterus, breast, prostate, liver, kidney, bone, and pancreas [1-11]. Ultrasound is attenuated and distorted by bone. Hence, noninvasive transcranial ultrasound treatment in the brain requires an adaptive focusing, taking into account the effects of the skull. This adaptive focusing can be achieved by using phased arrays with large surface area, the acoustic properties of the skull derived from high resolution CT scans, and high-performance computers to calculate the corrections necessary to restore a focus in the brain. Now this non-invasive trans-skull focusing for the purposes of thermal ablation in the brain has reached the early stages of clinical testing [12;13]. However, this technique has several limitations including the need for high power levels of ultrasound to penetrate the skull and skull heating that prevents ablation at many locations in the brain. Numerous experiments have demonstrated that ultrasound contrast (US) agents can reduce the power requirements for FUS ablation. Recently, we used 1.5 MHz FUS combined with Optison®, a commercially available contrast agent, and were able to produce localized lesions at greatly reduced power level [14;15]. Our experiments also showed that non-thermal mechanisms are important in such lesions. In this exploratory study, we investigated such ablation with exposures more suited for transcranial focused ultrasound.

#### METHODS

Animals. The study protocol was approved by the Animal Care Committee of Sunnybrook Research Institute. The rats were anesthetized with a mixture of sodium xylazine (Xyla-ject; Phoenix Pharmaceuticals, St Joseph, MO, USA) and ketamine hydrochloride (Abbott Laboratories, North Chicago, IL, USA). Overall, 48 locations in the brains of 12 rats (four non-overlapping locations/brain) were sonicated through the intact skull. Before the experiments, the hairs on the rat head were removed with hair clippers and depilatory lotion. The animals were sacrificed 24h (N=5), 48 h (N=3) or 7 days (N=3) after sonication. One animal died 5h after the treatment.

*Sonication.* The ultrasound fields were generated by an air-backed single focus transducer at 558 kHz (diameter/radius of curvature: 10/7.8 cm) (diameter/length of focal spot at half-maximum the pressure amplitude: 3mm/10mm). Ultrasound energy was delivered in pulsed sonications with burst length of 10msec, pulse repetition frequency (PRF) of 1Hz; total exposure 5 min. Additional experiments tested continuous-wave exposures (N=2) and bursts of 200ms (N=4) and 500ms (N=2). Four values of acoustic power of 0.47, 0.59, 0.89, and 1.3W (estimated peak pressure amplitude of 0.267, 0.300, 0.366, 0.444 MPa) were tested. Before each sonication, an US contrast agent, Definity (Lantheus Medical Imaging, N. Billerica, MA, USA) was injected intravenously (0.02 mL/kg).

*Experimental setup.* The experiments were performed in a clinical 1.5T MRI scanner (GE Healthcare, Milwaukee, WI) with the transducer housed in a mechanical positioning system and submerged in a tank of degassed, deionized water. The animals lay supine on a tray that was placed above this tank. A degassed water bag provided acoustic coupling between the transducer and the animal head.

*MRI*. MRI-based thermometry (proton resonant frequency shift method) was used to map the temperature rise during sonications. Contrast-enhanced T1-weighted MR images were acquired before and after injection of a MRI contrast agent (Omniscan, 0.1 mmol/kg) to visualize the extent of blood-brain barrier (BBB) opening. T2-weighted images were acquired after sonications to exam the development of edema, which is an indicator of local tissue damages.

*Histology.* Sections were stained with hematoxylin and eosin (H&E) for routine histological evaluation and with TUNEL staining (ApopTag, CHEMICON International, Inc., CA) for labeling DNA strand breaks that occur during apoptosis [16].

# RESULTS

In histology, a range of tissue effects were observed from no changes or mild vascular injury to lesions involving the whole sonicated region. At 24 and 48 hours, the most consistent feature was extravasation of red blood cells into the brain parenchyma and associated acute degeneration of the surrounding neurons. The minor damage (3/36) appeared as a few tiny red blood cell extravasation (petechiae) with either no change or small changes to the brain parenchyma. Eight of thirty six areas demonstrated scattered microscopic regions of perivascular hemorrhage and selective neuronal necrosis. Glial cells and most neurons appeared undamaged, and normal neurons might be seen adjacent to severely damaged cells (Figure 1a-c).

Twenty five sonicated areas demonstrated lesions presumably caused by a cessation of blood supply due to microbubble activity (cavitation) in the blood vessels. We observed lesions with moderate perivascular hemorrhage appearing to be predominately characterized by ischemia. At 24 h after sonication, these non-perfused areas were clear of tissue structure and contained only a few cell fragments and debris: there was autolysis resulting in the early formation of a semi-liquid mass of dead cells with mild inflammatory infiltration (Figure 1d-f). In other lesions with more extensive hemorrhages, tissue structures could still be discernible; these lesions were predominately characterized by hemorrhagic necrosis (Figure 1g-j). In some cases we observed almost complete destruction of the capillary blood vessels. In the most severe cases mechanical destruction of the brain parenchyma was present. The edges of the lesions were not well-defined and it was difficult to determine the full lesion extent. Several mm away from the apparent edge the lesions, additional hemorrhages were sometimes observed, especially at interfaces between different brain structures and in the ventricles.

Seven days after sonication, 4 of 12 locations displayed cellular infiltrates comprised of a mixture of astrocytes and macrophages; in one of these 4 locations small group of red blood cells still presented. Only sparse macrophages were found in 8/12 locations.

The lowest value for the production of lesions with 10ms/1Hz pulsed sonications and total exposure of 5 min, was 0.47W (estimated peak pressure amplitude of 0.267Mpa). The probability for lesion production was 50% (7/14). At acoustic power of 0.59W, 0.89W, and 1.3W (estimated peak pressure amplitude of 0.3MPa, 0.366MPa, and 0.444MPa), a probability of lesion production was ~ 100% (18/19).

In MRI, lesions appeared as enhancing regions in T2-weighted images as well as contrast-enhanced T1-weighted images. Good agreement was observed between the dimensions of the lesions in T2-weighted and contrast-enhanced T1-weighted imaging.

In summary, we demonstrated that US contrast agent can be combined with 558 kHz FUS to produce localized lesions in the brain at reduced acoustical power. The histological findings suggest that FUS combined with Definity produced localized lesions mainly through cavitation-induced damage to the blood vessels. In particular, we surmised that ultrasound combined with an ultrasound contrast agent might produce localized lesions through two cavitation-mediated processes: ischemia due to occlusion of the capillary blood vessels (through the formation of emboli and activation of platelet aggregation) and hemorrhagic infarction presumably caused by strong inertial cavitation damaging capillary blood vessels. The US contrast agent consists of preformed microbubbles that can act as nucleation promotion agents for cavitation. The cavitation microbubbles might obstruct blood flow in the capillary, thus causing tissue ischemia followed by inflammatory response and complement activation. Aggregation of platelets and clot formation might occur as well, leading to further obstruction of microcirculation and tissue damage. Microbubbles oscillation is

known to cause high shear stress, which leads to a wide range of effects on platelets from aggregation to destruction [17]. However, if the shear force is high, platelets can be destroyed liberating tissue factor, which plays an important role in the generation of thromboembolic complications [17;19;22]. Studies in animal models and humans demonstrated that the presence of gas microbubbles causes the pro-thrombotic effects through mechanisms that include increased platelet aggregation, complement-activation, fibrinolysis, release of tissue-factor, reduced endothelial function, and increased leukocyte infiltration [17-20].



*Figure 1.* Microphotographs of H&E stained sections demonstrating effects of ultrasound exposure in the brain parenchyma: (a)-(b) scattered microscopic regions of perivascular hemorrhage due to capillary blood vessel leaks into the brain parenchyma, which appears mostly undamaged; (c) a few affected neurons (arrows) can be seen adjacent to presumably damaged capillaries; (d)-(e) lesion with moderate hemorrhage appearing to be predominately characterized by ischemia: at 24h lesion is mostly free from both cells and any tissue structures; (f) dead neurons appear extremely

pale almost as "ghost" cells; (g)-(h) extensive hemorrhagic lesion (infarct); (j) necrotic neurons demonstrate loss of cell membranes and shrunken nuclei (arrows). Bars: a, d, g -1mm; h-0.5mm; b, e - 100μm; c, f, j -50 μm.

Using platelet-rich plasma, Poliachik et al. [21] demonstrated that high-intensity focused ultrasound (HIFU) activated platelets, stimulated them to aggregate and promoted their adherence to a collagen-coated surface. Cavitation was found to be a mechanism for platelet aggregation [22]. Furthermore, while aggregation did not occur without cavitation, excessive cavitation caused too much platelet damage to allow aggregation to occur.

We surmised that occlusion of blood vessels was presumably caused by stable cavitation; though sporadic inertial cavitation events also presented. Inertial cavitation (bubbles' collapse) generated high-speed microjets and high-pressure shock waves that were responsible for mechanical damage to the endothelial blood vessel walls. Strong inertial cavitation probably did not allow aggregation to occur, partially preserving blood flow in several capillaries. It might explain that despite extensive multiple perivascular hemorrhage some neurons appeared to survive. Despite the low power used, ultrasound-produced heating was observed in MRI thermometry acquired during the sonications. However, microbubbles caused susceptibility artifacts around the focus in MR thermometry images; therefore it was difficult to determine the exact shape of temperature distribution. Only the center of the focus was clearly depicted in the thermometry images. The temperature was not consistent in this study and sometimes it was hard to determine whether temperature values were real or due to measurement errors. For example, in 4/48, temperature di elevate more than 10 degrees, while in other rats with the same acoustic parameters the temperature increases were much lower.

This work demonstrates that ultrasound contrast agent can be combined with 558 kHz ultrasound to produce lesions in the brain. Future work will be necessary to optimize the exposures that can produce well-defined

ischemic lesions without complication of extensive hemorrhages. To precisely determine the volume of damaged tissue, different histological staining will be necessary.

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