Histological examination of focused ultrasound effects on human brain tissue

1. Introduction

Of recent interest in neuromodulation is the use of Low Intensity Focused Ultrasound [1] (FUS), itself a topic of significant interest and of several experiments conducted recently in humans with FUS [2,3]. The FDA Guidance on acoustic output for diagnostic ultrasound limits the derated spatial-peak temporal-average ultrasound intensity (ISPTA.3) to 0.72 W/cm². Much of the human neuromodulation work has been conducted at this limit [2]. However, as this threshold was established principally for diagnostic, as opposed to therapeutic ultrasound, some groups have conducted research at higher intensities [4], upwards of 6.16 W/cm² to understand better what range of intensities is most effective for neurostimulation. With higher intensities comes a greater risk of non-reversible injury to the tissue, such as thermal or mechanical damage including cavitation [5] and apoptosis [6]. With this in mind, we designed the present study to investigate the effects of focused ultrasound on human brain tissue to assess intensities at which focused ultrasound might cause irreversible structural cell damage. Because of the possibility that high ultrasound intensities might be destructive, we worked exclusively on excised tissue.

2. Methods

For our initial experiments, pieces of human brain tissue came from either a cadaver, or from tissue blocks resected during surgery. Specifically, cases #1–#5 were taken from recently deceased adult cadavers (less than 24 hours post-mortem), while cases #6–#9 were taken from surgical resections of adult patients undergoing temporal lobe resection to address medically intractable temporal lobe epilepsy. In all instances, the tissue came from the anteromesial temporal lobe. The tissue pieces were further cut into individual brain tissue samples approximately 1cm by 2cm wide, and 5–7mm in height, each one labelled with the case number and sample number (e.g. sample 3 from case 01 was denoted BTS01-3). Generally, there were seven to nine samples generated from each piece of tissue. In all, we used 61 individual samples that came from 9 separate patients, and each sample was treated at a single, distinct exposure condition as described below (or was sham for control purposes). The cadaveric samples were exposed to ultrasound immediately after extraction from the calvarium, while surgical resections were stored in Gibco Hibernate-A Medium (Thermo-Fisher Scientific; Waltham, MA) for the time it took to transport the samples from the OR to the laboratory, which was about 20–30 minutes.

We placed individual samples one at a time on a plastic petri dish that was on top of a chamber filled with degassed water (Fig. 1A). At the bottom of the chamber was the focused ultrasonic transducer (61.5mm diameter, 42mm focal length, 3.5mm focal beamwidth, 650kHz), positioned with the ultrasound focus at the center of the petri dish. With a focal distance of 42mm and a center frequency of 650kHz, the derating factor (to convert intensity values measured in water to derated) is 0.828. Based on separate measurements of the axial intensity profile, the intensity was essentially constant through the depth of the sample (within ±8%). The transducer was then connected to the ultrasound device (BX Pulsar 1002, BrainSonix Corp, Sherman Oaks, CA) using a coaxial cable. A separate experiment was performed to determine the effect of the petri dish on the ultrasound field, by placing a hydrophone at the position of the tissue sample, and measuring the intensity with and without the petri dish in place. The intensity loss was 3.2dB (31% pressure loss), which matches the theoretical loss through plastic from water to tissue.

All samples were sonicated for exactly 60 seconds. The table provides details of the acoustic exposure parameters, and each sample was subjected to one of exposure conditions from the list. In all instances, we included a control sample that was not sonicated, but that was subjected to the same handling procedures to serve as a comparison for the sonicated sample. During sonication, a plastic zip bag filled with castor oil was placed on top of the tissue block to absorb all ultrasound remnants that passed through the tissue. Immediately after sonication, we placed the tissue in formalin.

There were two exposure regimes, the first using pulsed excitation, and the second using continuous wave (CW) excitation. The table below summarizes the pulsed exposure conditions, taking into account the effect of the petri dish on the ultrasound field. The derated ISPTA.3 is provided for reference. Samples from subjects 1–3 (30 samples, all cadaveric) were exposed to pulsed excitation.

The 31 samples from subjects 4–9 (the first two cadaveric, the remainder fresh) were exposed to CW ultrasound at intensities (ISPTA.3) ranging from 1400 to 95,000 mW/cm², corresponding to peak rarefractional pressures (p0d) of 0.2–1.6MPa. Each sample was exposed at a single specific intensity.

After a period of 48 hours in formalin, we embedded the tissues in paraffin wax, sliced to 4 μm, and stained using hematoxylin and eosin. The slides were examined by a member of the investigative team (NK, SR), who was kept blinded to the coding (sonication vs control) of the blocks.
The main effects of ultrasound sonication investigated were signs of cell damage and death, including both apoptotic [7] and nonapoptotic (i.e. necrosis) [5] mechanisms, as well as cavitation.

3. Results

None of the samples sonicated using the pulsed regimes showed any adverse effects of ultrasound stimulation. Further no sample exposed to CW intensities below 14,000 mW/cm² ISPTA.0 (corresponding to 11,800 mW/cm² ISPTA.3) showed adverse effects of ultrasonic stimulation. Above this level, tissue began to show signs of spongiosis, albeit inconsistently, suggesting that the ultrasound had damaged the tissue (Fig. 1B and C).

4. Conclusion

Overall, FUS does not appear to cause significant heating or cavitation to brain tissue when ISPTA.0 remains below 14,000 mW/cm². It is important to remind readers that the FDA Guidance on acoustic output for diagnostic ultrasound limits the derated spatial-peak temporal-average ultrasound intensity (ISPTA.3) to 720 mW/cm². However, it becomes clear that ultrasound can be delivered at intensities above the FDA limit with no damage. FUS at ISPTA.0 below 14,000 mW/cm² appears to be safe both with regards to causing measurable cell damage or death, within the brain. However, as the tissue was fixed immediately post-sonication, there was not sufficient time for apoptosis to develop, leaving open the possibility that a lower intensity over a longer period of time may cause stress and damage to the cell, leading to its eventual death. There have been other studies which have sonicated for longer than the 1 minute sonication in this study [2,3]. However, those studies used a 5% duty factor, which means that the active sonication time is 5% of the sonication duration. As a reminder, most of the samples in this study were sonicated with 100% duty factor (continuous wave). For example, in the case of Badran et al. sonication was delivered in 10 30-s trains with 5% duty factor. As such, total active sonication was 15 seconds, which is four times less than what was delivered in this study for BTS04-BTS09. BTS01-03 were exposed to pulsed mode with lesser duty factors. While additional studies should continue to further ensure the safety of this novel and important technology, this furthers the notion that, at the current FDA Guideline levels, and potentially beyond, tFUS is likely a safe clinical technology.

| ISPTA.3 (mW/cm²) | ISPTA.0 (mW/cm²) | ISPPA.0 (W/cm²) | Pulse Duration (ms) | Pulse Repetition Frequency (Hz) | P0.0 (MPa) |
|------------------|------------------|-----------------|---------------------|--------------------------------|------------|
| 356              | 430              | 8.6             | 0.5                 | 100                            | 0.51       |
| 570              | 688              | 13.8            | 0.5                 | 100                            | 0.64       |
| 713              | 860              | 17.2            | 0.5                 | 100                            | 0.72       |
| 1425             | 1720             | 3.4             | 50                  | 10                             | 0.32       |
| 2850             | 3441             | 6.9             | 50                  | 10                             | 0.45       |
| 5700             | 6882             | 13.8            | 50                  | 10                             | 0.64       |
Declaration of competing interest

Dr. Alexander Korb is Vice-President of BrainSonix Corp and owns shares in the company. Dr. Alexander Bystritsky is the Founder and CEO of BrainSonix Corp. Dr. Mark Schafer is a consultant to BrainSonix Corp. Other authors report no conflicts of interest.

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