EFFECTS OF HIGH-INTENSITY FOCUSED ULTRASOUND WITH DIFFERENT ACOUSTIC DOSES ON NEURAL TISSUES *IN VITRO*

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1. INTRODUCTION

Replacing invasive surgical procedures with non-invasive, bloodless interventions can lead to significant advancements in the field of medicine. Therefore, development of non-invasive therapies is of utmost importance. By applying the physical principles of acoustics, high-intensity focused ultrasound (HIFU) has been introduced as a promising therapeutic modality due its capability to induce thermal and mechanical effects in deep-seated tissues of interest selectively and non-invasively.

Ultrasound-induced nerve conduction block has been investigated *in vitro* and *in vivo* [1-4]. Foley et al. (2004) created permanent nerve block and suppression of nerve function by thermally coagulating rabbit sciatic nerves *in vivo* using a 3.2-MHz HIFU transducer with a focal acoustic intensity of 1480 to 1850 W/cm^2 and 36 ± 14 (mean± SD) seconds of sonication [4]. Using a 3.5-MHz ultrasound transducer, the effects of unfocused, continuous wave ultrasound on *in vitro* frog sciatic nerves at three different acoustic power levels (1, 2 and 3 W) was studies for a sonication time of 5 minutes [1]. Ultrasound exposure at acoustic power of 1 W resulted in nerve stimulation by increasing the compound action potential (CAP) amplitude by 8%. A progressive decrease in the nerve CAP amplitude was observed for 2 and 3 W ultrasound exposures [1].

Although the aforementioned studies have investigated the effects of focused and unfocused ultrasound with different acoustic power levels or intensities on nerve conduction *in vivo* and *in vitro*, little is known about the combined effects of acoustic intensity and sonication time on nerve conduction. The product of the acoustic intensity and sonication time yields a treatment parameter that was termed acoustic dose [5]. The goal of this study is to investigate the dose-dependent biologic effects of HIFU on lobster's ventral nerve cord *in vitro*.

2. MATERIALS AND METHODS

A spherically concaved HIFU transducer with resonance frequency of 2.2 MHz was utilized. The aperture diameter of the transducer is 5.0 cm and its radius of curvature is 7.5 cm. Thus, the f-number of this transducer is 1.5. The HIFU transducer was brought in close proximity to the target tissue. Depending on the acoustic dose level, two tissue targeting methods were utilized in this study.

2.1 Low- and medium-level acoustic doses

For low-and medium-level acoustic doses treatments, a ventral nerve cord was excised from a marine lobster (*Homarus Americanus*) and placed on a nerve chamber to measure its CAP before and after exposure to HIFU using an electrophysiology system (BIOPAC Systems, Inc., Goleta, CA) connected to a computer. Droplets of Ringer's solution (462 mM NaCl, 16 mM KCl, 26 mM $CaCl_2$, 8 mM $MgCl_2$, 10 mM tris, 10 mM maleic acid, and 11 mM glucose) were provided to the neural tissue to supply vital nutrients and ions.

Because the HIFU transducer is strongly focused, both low and medium-level acoustic doses were achieved by placing the neural tissue in the pre-focal region of the HIFU transducer. Using a linear acoustic and temperature simulation (LATS), the spatial-peak temporal-average intensity (I_{SPTA}) was determined [6]. In the low-level acoustic dose treatment, the I_{SPTA} was around 3.3 W/cm^2 and the sonication time was 10 seconds (i.e. acoustic dose of 32.5 J/cm^2). In the medium-level acoustic dose treatment, the I_{SPTA} was around 13.3 W/cm^2 and the sonication time was 10 seconds (i.e. acoustic dose of 132.6 J/cm^2).

2.2 High-level acoustic dose

For treatments with high levels of acoustic dose, the lobster's ventral nerve cord was sandwiched between the nerve chamber and an *in vitro* chicken breast tissue, a scenario that resembles *in vivo* experiments. A SONIX RP® clinical ultrasound imaging system (Ultrasonix Medical Corp., Richmond, BC, Canada) was utilized to monitor treatment in real time pre-, during and post-exposure to HIFU therapy via its EC4-9/10 R endocavity imaging probe (Ultrasonix Medical Corp., Richmond, BC, Canada). The imaging probe was aligned with the therapeutic HIFU transducer such that the focal zone of the therapeutic transducer appears on the screen of the ultrasound imager, thereby guiding the acoustic therapy to the target (i.e. the neural tissue). Similar to the previous treatments, Ringer's solution was supplied to the neural tissue. The sonication time for high-level acoustic dose therapy was 5 seconds and the I_{SPTA}, as determined by LATS, was around 5500 W/cm^2 (i.e. acoustic dose of 27500 J/cm^2).

3. RESULTS

Results of the HIFU exposures to neural tissues at the three difference acoustic dose levels are shown in figure 1 and summarized in table 1.

At low-level acoustic dose, the nerve CAP amplitude increased by 18.0% after a 10-second HIFU exposure of around 3.3 W/cm^2. At medium-level acoustic dose, the nerve CAP amplitude decreased by 5.4% following a 10-second HIFU exposure of around 13.3 W/cm^2. A greater suppression in the nerve CAP amplitude was achieved at high-level acoustic dose. A 5-second HIFU exposure of around 5500 W/cm^2 resulted in a 57.8% decrease in the nerve CAP amplitude. Moreover, gross examination of the chicken breast and neural tissues subjected to the high-level acoustic dose reveals a discoloration and coagulative necrosis in a localized volume where both tissues meet.

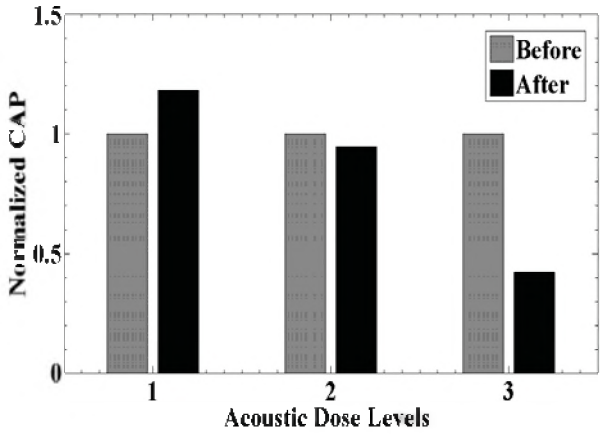

Figure 1. A comparison of the normalized nerve CAP amplitudes before and after HIFU treatments at the three different acoustic dose levels (1: low-level acoustic dose; 2: medium-level acoustic dose; 3: high-level acoustic dose).

Table 1. Summary of the results

	Low-level acoustic dose	Medium-level acoustic dose	High-level acoustic dose
Relative effect of HIFU on CAP amplitude	Increase by 18.0%	Decrease by 5.4%	Decrease by 57.8%

4. DISCUSSION AND CONCLUSIONS

Decrease in the nerve CAP amplitude after HIFU treatment at high and medium acoustic dose levels demonstrates the ability of HIFU to induce nerve conduction block primarily due to its thermal mechanism, which is more pronounced at high acoustic dose levels. The thermal effect of HIFU therapy has been previously shown to be responsible for conduction block of frog sciatic nerve *in vitro* [3]. The ultrasound-induced reduction of the CAP amplitude has been attributed to the ability of ultrasound's thermal effect to partially disable the axonal ion channels of the nerve, reducing the number of ions (Na^+ and K^+) passing through the axonal membranes and thus decreasing the nerve CAP amplitude [1]. On the other hand, increase in the nerve CAP amplitude after HIFU treatment at low-level acoustic dose demonstrates the ability of HIFU to stimulate neural tissues primarily due to its mechanical mechanism (non-thermal effect). By producing a change in their membrane potential, the mechanical force of HIFU therapy has been proposed to be responsible for stimulating neural structures [7]. Ultrasound-induced nerve stimulation, as evidenced by an increase in the nerve CAP amplitude, has recently been attributed to the opening of the axonal ion channels with the mechanical stimulation of ultrasound, allowing more ions to pass through the axonal membranes and thereby augmenting the nerve CAP amplitude [1].

Results of this study demonstrate the great advantage of HIFU as a non-invasive and localized acoustic therapy with promising applications in neurology, neurosurgery, and anesthesiology and pain management [8].

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