Investigations into the potential contribution of a thermal mechanism for pulsed high intensity focused ultrasound mediated delivery

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***Abstract*—The mechanism behind pulsed high intensity focused ultrasound (pHIFU) effects leading to increased drug delivery is currently poorly understood. In this work, the thermal dose and peak temperatures associated with a typical pHIFU treatment were measured in mouse muscle. A non-ultrasonic hyperthermia (HT) treatment was then applied, designed to mimic the thermal component of the pHIFU treatment. The delivery of 200 nm fluorescent nanoparticles was measured as a surrogate marker for drug delivery by pHIFU and HT treatments. Only the pHIFU treatment showed a significant increase in particle delivery.**

Keywords—drug delivery; high intensity focused ultrasound; hyperthermia; thermal mechanism

I. INTRODUCTION

Although ultrasound mediated drug delivery is not a new concept [1], it typically relies on cavitation effects or is applied as a form of hyperthermia [2,3]. For some time now, we have been reporting on the use of a pulsed-mode HIFU (pHIFU) drug delivery designed to avoid both cavitation and sustained high temperatures [4,5,6]. The goal is to avoid tissue damage while still delivering locally increased levels of therapeutics, ostensibly via an alternative mechanism. Despite the uncertainties of this approach, we have consistently been able to show significant therapeutic benefits in the delivery of various agents to animal muscle, tumor, and even blood clots.

In this study, the goal was to isolate the effect of the thermal component of a pHIFU treatment and directly compare it to the full thermal plus mechanical pHIFU. We have shown that our typical pHIFU treatment delivered a peak temperature increase of a little less than 5 ˚C (just under 42 ˚C when using a bath temperature of 37 ˚C) [4]. Furthermore, this temperature is sustained, in a given treatment location, for less than 2 minutes. In contrast, a typical hyperthermia treatment at 39-42 ˚C may require up to an hour before significant effects occur [7]. Nevertheless, a conclusive comparison must be made using similarly controlled measurements of the effect. Here, we employed a histological technique for assaying the delivery of fluorescent nanospheres throughout the mouse muscle. The same sections could then be used to look at morphological changes. As another test of the thermal component, we also compared to low temperature pHIFU treatments, cooling the tissue temperature by 3 degrees to bring the peak temperature below 39 ˚C, thus avoiding hyperthermia altogether.

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II. MATERIALS AND METHODS

A. pHIFU Treatment

A custom HIFU system was used for our study, modified from a Sonoblate® 500 (Focus Surgery; Indianapolis, IN)*. The probe possesses both a therapeutic (1 MHz) transducer and a collinear imaging (10 MHz) transducer, each with a focal length of 4 cm. The therapeutic transducer has a focusing factor of 1.3×10^3, with an acoustic output calibrated using the radiation force technique. The focal zone has an axial length of 7.2 mm and a radial diameter of 1.38 mm. A single exposure consists of 100 pulses at a pulse repetition frequency of 1 Hz, 5 % duty cycle and a peak total acoustic power (TAP) of 40 W, unless otherwise specified. A full treatment of the mouse calf consists of six single exposures at a 2 mm separation in a 2 x 3 grid pattern. Standard treatments (pHIFU37) are carried out in a bath with temperature controlled to 37 ˚C (see Fig. 1). The low temperature (pHIFU34) treatments were carried out with the bath temperature reduced to 34 ˚C.

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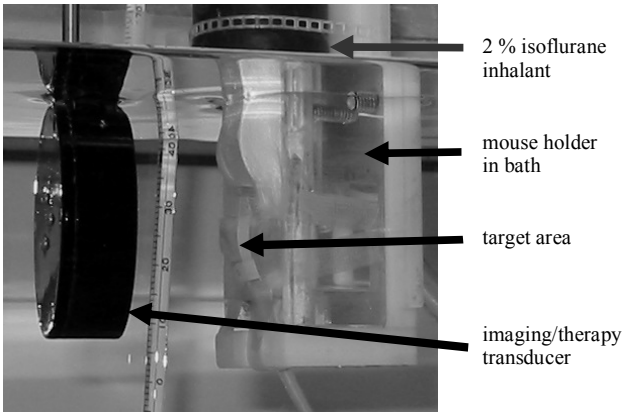

Figure 1. Pulsed HIFU treatment of mouse flank in a water bath.

All animal work was performed according to an approved animal study protocol and in compliance with NIH, Clinical Center Animal Care and Use Committee guidelines. Female C3H mice, at least eight months in age, were used for all experiments. Treatments were carried out as previously described [4,5]. Briefly, anesthetized mice (2 % isoflurane) were immersed, strapped in an upright position, in a degassed water bath. Targeting of the calf was achieved by imaging with the 10 MHz imaging probe. The full treatment described above requires 10 minutes to complete.

B. Measurement of the Thermal Dose

The thermal dose supplied by pulsed HIFU exposures was measured by a pair of 30 Ga hypodermic thermocouples (Omega Engineering Inc.; Stamford, CT). The temperature was sampled at 0.25 s intervals by a digital oscilloscope. The use of thermocouples suffers from one major drawback, that is, the difficulty of controlling the position of the thermocouple relative to the ultrasonic beam. Unfortunately, the ultrasonic guidance built into the SB500 has sufficient resolution for only a gross localization. To avoid problems due to misplacement of the probe [8], it is better to make use of measurements from several points outside of the focal zone, which might be extrapolated to find the true peak based on some theoretical assumptions. A series of complete thermal trajectories, including a full cool-down period, was measured during pulsed-HIFU treatments made on a known grid near and around a single probe insertion point. This process results in a sparse sampling of the spatial temperature distribution. The data were then fit to a cylindrically symmetric solution to the bio-heat equation in space and time [9], including the 'true' coordinates of the probe as additional fitting parameters. Peak temperatures were found to be from 3.5 to 4.5 ˚C above ambient. The profiles and peak temperatures were very similar to those reported previously in rabbit muscle, using the same exposure parameters and measured with MR-thermometry [10].

Since a full treatment typically does not allow for a cool-down period between grid points, heating from neighboring points is not insignificant. To practically measure the additional heating, a pair of thermocouples was used to record the temperature at two different locations during a continuous treatment without cooling. Of six such measurements, two resulted in recorded temperatures spiking one or more degrees beyond 43 ˚C at a single raster point, and were subsequently discarded as artifacts of direct probe heating. The others showed heating only slightly different from the previously extrapolated value.

From the temperature vs. time data, the T43 equivalent thermal dose was calculated using the formula from Dewey, for temperatures between 37 and 43 ˚C [11]. This was done for the fitted single point data, which was extrapolated to a full treatment by considering a serial contribution from nearest neighbor raster points. It was also calculated for the temperature data collected during the continuous treatment. The hyperthermia treatment (HT) was then designed to be greater than the pHIFU37 heating both in terms of temperature and thermal dose (Fig. 2). The thermal dose of the pHIFU34 treatment is negligible.

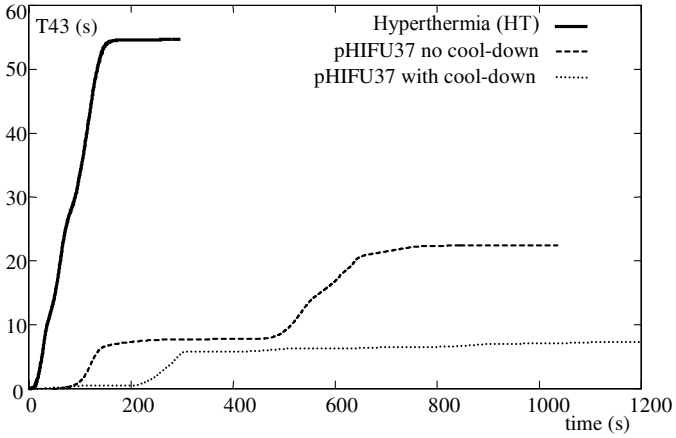

Figure 2. T43 thermal doses calculated for pHIFU treatments; comparison with HT treatment.

C. Heat Treatment

The HT consisted of the thermal dose described above administered with a heat lamp powered through a potentiometer (Fig. 3). The lamp was placed approximately 1 cm above the target limb. The core of the animal and the control limb were covered by a 'blanket' of gauze to prevent unintentional heating. Due to the uncertainties involved in this process, the treatment was monitored with the same hypodermic thermocouples previously used to measure the pHIFU thermal dose. A similar thermocouple was located in the control limb. The heat lamp output was continuously fine-tuned to meet and maintain the target temperature of 42 ˚C. Following two full minutes at this temperature, the heat lamp was turned off and removed to allow the limb to cool as rapidly as possible. It should be noted that the ambient temperature of the exposed limb, even on a heated pad, was 34-35 ˚C, significantly less than the pHIFU37 bath temperature. Also, the heating of the blood pool is not insignificant in the case of the HT, and the control limb temperature typically rose by 1-2 ˚C. In contrast, significant heating of the control limb does not occur during pHIFU treatments.

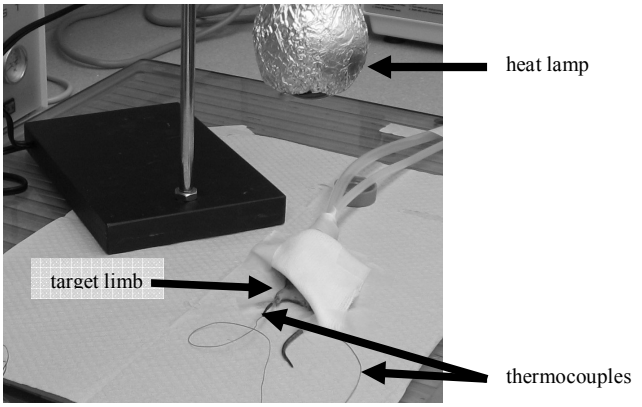

Figure 3. HT treatment setup. Heating in the limb is monitored with interstitial hypodermic thermocouples and manually adjusted by controlling the current through the heat lamp.

D. Nanoparticle Delivery Assay

Following treatment, each mouse was allowed to recover from anesthesia for approximately 20 minutes prior to receiving a tail vein injection of 100 µl of stock solution 200 nm diameter red fluorescent microspheres (ex: 580 nm; em: 605 nm, Invitrogen Corp.; Carlsbad, CA). Unless otherwise mentioned, the particles were allowed to circulate for one hour before sacrificing the mouse. Following sacrifice, the remaining particles in the vasculature were flushed by perfusing with saline. This technique was used in lieu of waiting for the bloodstream to clear normally, and was validated against waiting a clearing period of 24 hours following injection.

After sacrifice and perfusion, the treated calf muscle was extracted and frozen. Cryosections were taken perpendicular to the ultrasonic treatment axis from regular depths at 1 mm intervals, beginning at 0.5 mm from the treatment surface and progressing to the far side of the leg, typically 5.5 mm. Sections were viewed unstained under a fluorescent microscope. Images were collected, for each section, on a 2x2 mm grid, at 100× magnification and a resolution of 3900x3090 pixels. Typically 5 to 8 images could be collected at each depth, depending on the size of the section, for a total of 25 to 40 images per leg. These became the raw data for further processing and analysis. A few cross-section and surface images were also collected for qualitative analysis.

E. Image Processing

Using thresholding and particle analysis routines included in ImageJ (NIH), the area fraction of the "fine" particles was measured. Particles greater than 300 pixels in size were excluded from consideration by removing their area from both numerator and denominator of the calculation. This approach removed the vast majority of the obvious artifacts, as well as crudely weighting in favor of better particle distribution. Typically, the large particle area fraction represented less than 1 % of the total image area. The measured quantity was thus the net "fine particle density" = (sum of "fine particle" area)/(sum of (image area – artifact area)) for all images collected from a given sample.

A non-parametric, paired test (Mann-Whitney) was used to determine the significance of the difference between net fine particle densities measured in control and pHIFU37 treated legs (N=6), control and pHIFU34 treated legs (N=7), and between control and HT treated legs (N=6).

III. RESULTS

In the "standard" pHIFU37 treated muscle, the median fine particle density (MPD) was found to be 0.060 % (Fig. 4). This was a significant increase (p=0.016) over the control muscle density of 0.030 %. The pHIFU34 treated muscle showed an even larger difference, with a MPD of 0.18% in the treated side vs. 0.028 % in the control (p=0.008). Finally, in the HT animals, the MPD was 0.033 %, which is not significantly different (p=0.34) from that of the control at 0.027 %.

Besides the quantitative analysis, a number of images were collected for qualitative analysis. Cross-sections and skin surface sections showed a much higher distribution of particles at the skin surface and just below, to a depth of less than 0.2 mm, than that found in the bulk tissue. It was also noticed that a large deposit of these particles could always be found in the bone marrow, and care had to be taken to prevent this from unduly contaminating other portions of the slide whenever the sectioning knife sliced through bone. Many of our conclusions are dependent, to a certain extent, on the assumption that skin and bone effects are too small and localized to affect a significant fraction of the muscle.

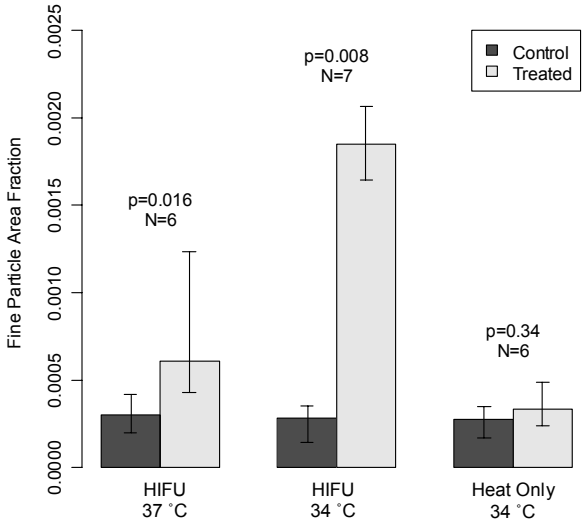

Figure 4. Comparison of 200 nm microsphere delivery following pHIFU treatment at high and low bath temperatures and HT treatment

IV. DISCUSSION

There was little doubt, going into this study, that the pHIFU delivery effects were not strictly heat related, given the large disparity in heating times and spatial extent between typical pHIFU and hyperthermia treatments. It was not so clear that we

would be able to demonstrate this using a global heat source such as a bath or heat lamp rather than a focal source more like a pHIFU exposure. The HT described here heats the entire muscle, representing a significant fraction of the animal's blood pool, and therefore would be expected to generate a much larger physiological effect than the pHIFU treatment. Also, because the pHIFU treated region, representing a region of 4 mm by 6 mm, is smaller than the muscle area, it is difficult to be certain that the resected tissue corresponds precisely to the treated area. This is obviously not a problem during HT. Despite these handicaps, only the pHIFU treatments showed any increase in particle distribution, providing convincing proof that temperature alone cannot possibly explain the effect. More surprising, perhaps, is the effect of lowering the bath temperature from 37 ˚C to 34 ˚C during pHIFU treatment. If the effect were due to hyperthermia, this would be expected to result in a very significant reduction in delivery. Instead, there is a quite significant increase. This unexpected result will require further study to understand. One hypothesis is that the 37 degree bath is actually overheating the mice to a certain extent. Certainly the temperature of their extremities, measured prior to the HT treatment, is 2 to 3 degrees lower than this. As a result, when in the warm bath, perfusion patterns may be altered to compensate, effectively shunting nanoparticles to other locations. This theory would be more convincing if there were a significant change in the control limb as well.

If the pHIFU effect is not due to the temperature increase, the conventional wisdom would be that it is most probably due to cavitation. Indeed many groups looking for similar results deliberately induce cavitation by employing ultrasound contrast agents [2]. We do not, however, and it remains unclear whether or not cavitation plays a major role in this effect. A big part of the problem lies in the mouse geometry. The axial length of the ultrasonic focus (7 mm) is on the same order as the thickness of the mouse muscle. Thus it is impossible to avoid significant acoustic power at surface of the skin and femur. As the skin surface, in particular, has a quite low cavitation threshold, it is difficult to separate this effect from the more interesting effects occurring in the bulk tissue in a spectrum, for example. Indeed spectra recorded during treatments usually contained cavitation signatures such as increases in harmonics, half harmonics and broadband noise. On the other hand, no clear changes in echogenicity were ever observed; however, this doesn't entirely preclude the possibility of small or transient bubbles. Histology has consistently shown very little damage, such as micro-hemorrhage, that might be typically associated with cavitation effects [2]. Taken all together, this leaves a rather confused picture regarding the possibility of a cavitation based mechanism. Future investigations involving larger animal models will address these issues.

Other possible mechanisms that also might play a role include purely mechanical ones such as radiation force induced tissue shear or vascular microstreaming, as well as those that depend heavily on cellular signaling such as gene upregulation and cytoskeletal remodeling.

* The full technical description of the procedures used in this paper requires the identification of commercial products and their suppliers. The inclusion of such information indicates neither endorsement of such products and suppliers by us nor recommendations of superior suitability for the purposes described.

[1] K. Tachibana and S. Tachibana, "Emerging Technologies Using Ultrasound for Drug Delivery," in Emerging Therapeutic Ultrasound, J. Wu and W. Nyborg, Eds. Singapore: World Scientific Publishing, 2006.

[2] N. McDannold, N. Vykhodtseva, and K. Hynynen, "Targeted disruption of the blood-brain barrier with focused ultrasound: association with cavitation activity," Phys. in Med. and Biol., Vol. 51, pp. 793-807, 2006.

[3] P J. Benkeser, L. A. Frizzell, S. A. Goss and C. A. Cain, "Analysis of a Multielement Ultrasound Hyperthermia Applicator," IEEE Trans. Ultrasonics, Ferroelectrics and Frequency Control, UFFC-36, pp. 319-325, 1989.

[4] V. Frenkel, C. Deng, B. E. O'Neill, *et al.* "Pulsed-high intensity focused ultrasound (HIFU) exposures for enhanced delivery of therapeutics: mechanisms and applications," in Proc. of the 5th Int. Symp. on Therapeutic Ultrasound, G. T. Clement, N. J. McDannold, and K. Hynynen, Eds. Melville NY: AIP, 2006.

[5] K. M. Dittmar, J. Xie, F. Hunter, C. Trimble, M. Bur, V. Frenkel, and K. C. P. Li, "Pulsed high-intensity focused ultrasound enhances systemic administration of naked DNA in squamous cell carcinoma model: initial experience," Radiology, Vol. 235, pp. 541-546, 2005.

[6] M. J. Stone, V. Frenkel, S. Dromi, P. Thomas, R. P. Lewis, K. C. P. Li, M. Horne 3rd, B. J. Wood, "Pulsed-high intensity focused ultrasound enhanced tPA mediated thrombolysis in a novel in vivo clot model, a pilot study," Thromb. Res., in press.

[7] G. Kong, R. D. Braun, and M. W. Dewhirst, "Hyperthermia Enables Tumor-specific Nanoparticle Delivery: Effect of Particle Size", Cancer Res., Vol. 60, pp. 4440-4445, 2000.

[8] R. L. Clarke and G. R. ter Haar, "Temperature rise recorded during lesion formation by high-intensity focused ultrasound," Ultrasound Med. Biol., Vol. 23, pp. 299-306, 1997.

[9] M. L. Palmeri and K. R. Nightingale, "On the Thermal Effects Associated with Radiation Force Imaging of Soft Tissue," IEEE Trans. Ultrason., Ferroelect., Freq. Contr., Vol. 51, pp. 551-565, 2004.

[10] A. W. Kam, H. Wang, K. Farahani, D. Thomasson, B. O'Neill, M. Angstadt, J. Jesson, K. C. P. Li "Safety of Pulsed High Intensity Focused Ultrasound for Enhanced Drug and Gene Delivery", Proc. of the 6th Int. Symp. on Therapeutic Ultrasound, C. Coussios, G. ter Haar, Eds. Melville NY: AIP, 2007.

[11] S. A. Sapareto, W. C. Dewey, "Thermal dose determination in cancer therapy," Int. J. Radiat. Oncol. Biol. Phys. 10, pp. 787–800, 1984.