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Cavitation-enhanced MR-guided focused ultrasound ablation of rabbit tumors in vivo using phase shift nanoemulsions

Jonathan A Kopechek¹, Eun-Joo Park², Yong-Zhi Zhang², Natalia I Vykhodtseva², Nathan J McDannold², and Tyrone M Porter^{1,3}

¹Dept. of Mechanical Engineering, Boston University, Boston, MA, USA

²Dept. of Radiology, Brigham and Women's Hospital, Harvard Medical School, Boston, MA, USA

³Dept. of Biomedical Engineering, Boston University, Boston, MA, USA

Abstract

Advanced tumors are often inoperable due to their size and proximity to critical vascular structures. High intensity focused ultrasound (HIFU) has been developed to non-invasively thermally ablate inoperable solid tumors. However, the clinical feasibility of HIFU ablation therapy has been limited by the long treatment times (on the order of hours) and high acoustic intensities required. Studies have shown that inertial cavitation can enhance HIFU-mediated heating by generating broadband acoustic emissions that increase tissue absorption and accelerate HIFU-induced heating. Unfortunately, initiating inertial cavitation in tumors requires high intensities and can be unpredictable. To address this need, phase-shift nanoemulsions (PSNE) have been developed. PSNE consist of lipid-coated liquid perfluorocarbon droplets that are less than 200 nm in diameter, thereby allowing passive accumulation in tumors through leaky tumor vasculature. PSNE can be vaporized into microbubbles in tumors in order to nucleate cavitation activity and enhance HIFU-mediated heating. In this study, MR-guided HIFU treatments were performed on intramuscular rabbit VX2 tumors *in vivo* to assess the effect of vaporized PSNE on acoustic cavitation and HIFU-mediated heating. HIFU pulses were delivered for 30 seconds using a 1.5 MHz, MR-compatible transducer, and cavitation emissions were recorded with a 650-kHz ring hydrophone while temperature was monitored using MR thermometry. Cavitation emissions were significantly higher ($P < 0.05$) after PSNE injection and this was well correlated with enhanced HIFU-mediated heating in tumors. The peak temperature rise induced by sonication was significantly higher ($P < 0.05$) after PSNE injection. For example, the mean percent change in temperature achieved at 5.2 W of acoustic power was $46 \pm 22\%$ with PSNE injection. The results indicate that PSNE nucleates cavitation which correlates with enhanced HIFU-mediated heating in tumors. This suggests that PSNE could potentially be used to reduce the time and/or acoustic intensity required for HIFU-mediated heating, thereby increasing the feasibility and clinical efficacy of HIFU thermal ablation therapy.

Keywords

MR-guided high intensity focused ultrasound; Phase-shift nanoemulsions; Acoustic droplet vaporization; Microbubble cavitation; Tumor ablation

1. Introduction

Cancer is the second leading cause of death globally (Hoyert and Xu, 2012). Complete surgical resection of solid tumors is generally the only curative treatment available, but many advanced tumors are unresectable due to their size and proximity to critical vascular structures (Christoforidis *et al.*, 2002). For example, less than 30% of patients with liver tumors and less than 20% of patients with pancreatic tumors are eligible for complete surgical resection (Govaert *et al.*, 2012; Morganti *et al.*, 2010). To address this clinical need, a number of methods have been developed to ablate inoperable solid tumors.

Radiofrequency ablation and cryoablation are methods available clinically for destroying tumors by heating or freezing, respectively, in a localized manner. However, both treatment modalities require invasive insertion of probes directly into the tumors which carries a risk of complications (Jaskolka *et al.*, 2005). Furthermore, the probes can only destroy a defined volume around the probe, thus multiple insertions are required to treat large tumors (generally > 3 cm) or multiple tumors in different regions (Cabassa *et al.*, 2006). High intensity focused ultrasound (HIFU) has been developed as an alternative to these minimally invasive methods for complete ablation of inoperable solid tumors (Tempany *et al.*, 2011). Unlike minimally invasive modalities, HIFU can be focused through the skin, delivering sufficient acoustic energy to ablate small volumes (generally less than 10 mm³) within solid tumors without appreciable thermal damage to intervening tissue. Complete ablation is achieved by mechanically or electronically steering the HIFU beam through the solid tumor (Kennedy, 2005). In addition, magnetic resonance imaging (MRI) guidance enables accurate localization of the ultrasound focus throughout the tumor as well as real-time thermal mapping during treatment (Tempany *et al.*, 2003).

HIFU ablation therapy has been used clinically to treat a variety of malignancies, including tumors in the breast (Wu *et al.*, 2003a; Wu *et al.*, 2005a), pancreas (Wu *et al.*, 2005b), liver (Illing *et al.*, 2005; Kennedy *et al.*, 2004), kidney (Illing *et al.*, 2005; Wu *et al.*, 2003b), and prostate (Poissonnier *et al.*, 2007; Sanghvi *et al.*, 1999; Uchida *et al.*, 2002), and is FDA-approved for treating uterine fibroids and bone metastases in the United States (Hesley *et al.*, 2013). However, the treatment modality remains under-utilized clinically due to the long exposure times and high acoustic intensities required for debulking solid tumors completely (Fischer *et al.*, 2010). Studies have demonstrated that acoustic inertial cavitation can enhance the rate of heating in tissue-mimicking hydrogels (Farny *et al.*, 2010). This is primarily due to the fact that inertial cavitation emits acoustic energy at higher frequencies, which are more readily absorbed by tissue and converted into heat (Coussios *et al.*, 2007). Prior studies have introduced microbubbles in order to nucleate cavitation activity and enhance HIFU-mediated heating (Chung *et al.*, 2012; He *et al.*, 2011; Kaneko *et al.*, 2005; Luo *et al.*, 2009; McDannold *et al.*, 2006; Umemura *et al.*, 2005; Tung *et al.*, 2006). However, microbubbles are too large to extravasate and thus will be expected to enhance

heating only in and around blood vessels. In addition, microbubbles rapidly disappear from circulation, thus requiring multiple infusions during treatment. Sokka, et al. (2003) produced endogenous bubbles directly from the tissue, but this required very high powers and the density and activity of the resultant bubbles was likely highly variable due to the heterogeneity of different tissues (Sokka *et al.*, 2003).

To address these challenges of initiating inertial cavitation *in vivo* for bubble-enhanced heating and ablation, phase-shift nanoemulsions (PSNE) are being developed as an *in situ* cavitation nucleation agent (Kopechek *et al.*, 2012). PSNE consist of lipid-coated liquid perfluorocarbon (PFC) droplets that are less than 200 nm in diameter (Figure 1), allowing them to passively accumulate in tumors through the enhanced permeability and retention (EPR) effect (Zhang *et al.*, 2013). The liquid perfluorocarbon has a low boiling point, thus enabling the liquid droplets to be vaporized into gas-filled microbubbles *in situ* using short, high-amplitude acoustic pulses. Liquid perfluorocarbon droplets have been investigated for a range of potential therapeutic applications. Rapoport, et al. (2011) used a block co-polymer shell to co-encapsulate paclitaxel and perfluorocarbon droplets and investigated ultrasound-mediated delivery after vaporization (Rapoport *et al.*, 2011). Fabiilli, et al. (2010) delivered a lipophilic chemotherapeutic using albumin-coated perfluorocarbon emulsions and also produced a water/perfluorocarbon/water double-emulsion to deliver water-soluble compounds (Fabiilli *et al.*, 2010a; Fabiilli *et al.*, 2010b). Also, Zhang, et al. (2010) used acoustic droplet vaporization for vascular occlusion in canine kidneys (Zhang *et al.*, 2010). Williams, et al. (2013) described the use of sub-micron perfluorocarbon droplets to enhance ultrasound imaging contrast in tumors (Williams *et al.*, 2013). Furthermore, Strohm, et al. (2011) demonstrated optically-triggered vaporization of perfluorocarbon droplets by incorporating optical absorbing particles into the droplets (Strohm *et al.*, 2011).

Studies have shown that the microbubbles formed from vaporized perfluorocarbon emulsions can potentially nucleate acoustic cavitation and enhance focused ultrasound-mediated heating in tissue-mimicking phantoms (Zhang and Porter, 2010). Moreover, it has been shown that acoustic vaporization of micron-sized PFC emulsions injected intravenously while treating canine livers with HIFU significantly increased the volume of ablated tissue (Zhang *et al.* 2011). As the study was performed in a healthy canine liver, it has yet to be demonstrated that vaporized PFC emulsions can enhance HIFU-mediated ablation in solid tumors.

The goal of this study was to evaluate the effect of PSNE vaporization on the nucleation of acoustic cavitation and enhancement of HIFU-mediated heating and ablation in rabbit tumors *in vivo*. Cavitation activity and heating in the tumor were monitored using a cavitation detector and MR thermometry, respectively. After treatment, tumors were removed and histological analysis was performed. The information obtained in this study is needed in order to evaluate the potential role of PSNE in enhancing high intensity focused ultrasound-mediated thermal ablation of tumors *in vivo*.

2. Methods

2.1 Synthesis of Phase-Shift Nanoemulsions

The lipids 1,2-dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[amino(polyethylene glycol)-2000] (DSPE-PEG2000) were purchased from Avanti Lipids (Alabaster, AL, USA) and liquid perfluorocarbon (perfluoropentane) was obtained from Fluoromed (Round Rock, TX, USA). Lipids (190 mg) were dissolved in chloroform in a molar ratio of 94:6 DPPC:DSPE-PEG2000. The solvent was evaporated, and the dry lipid film was rehydrated with 10 ml phosphate-buffered saline. The lipid solution was sonicated for 2 minutes at 20% power using a Vibra-Cell sonicator probe (Sonics & Materials, Newtown, CT, USA) before adding liquid perfluoropentane (C_5F_{12}) at a volume ratio of 4%. The mixture was sonicated at 25% power in pulsed mode (10 s on, 50 s off) in an ice-water bath for a total sonication time of 60 s, followed by 16 passes through 200 nm polycarbonate membrane filters (Whatman, Piscataway, NJ, USA) using a LIPEX extruder (Northern Lipids, Burnaby, BC, Canada). The mean size and polydispersity of the droplets measured using a particle size analyzer (90Plus Particle Size Analyzer, Brookhaven Instruments, Holtsville, NY, USA) was 169 ± 22 nm and 0.17 ± 0.05 , respectively. The resulting PSNE solutions were stored at 4 °C until use.

2.2 Animals

All animal experiments were performed according to protocols approved by the Harvard Medical School Institutional Animal Care and Use Committee. VX2 tumors were established in the left thigh of 3 kg New Zealand white rabbits (Millbrook Breeding Labs, Amherst, MA, USA) by direct injection of 3×10^7 VX2 cells at a depth of approximately 1 cm. Tumors were allowed to grow for two weeks before experiments were conducted. During each procedure (surgery, ultrasound, or both), animals were anesthetized with a mixture of ketamine (40 mg/kg body weight, Aveco, Fort Dodge, IA, USA) and xylazine (10 mg/kg body weight, Lloyd Laboratories, Shenandoah, IA, USA) injected as an intramuscular bolus each hour. The body temperature was monitored throughout each experiment using a rectal thermocouple. Two hours before sonication, PSNE solutions (0.5 ml/kg body weight) were injected intravenously through a catheter in the ear vein and flushed with 0.5 ml saline. Animals were sacrificed at the end of each experiment and tumors were harvested for histological analysis of deposited thermal lesions. The results in this study were obtained from three animals. Each tumor was 1.5–2.0 cm in diameter.

2.3 Experimental Setup

A custom-built, MR-compatible HIFU transducer (1.5 MHz center frequency, 10 cm aperture, and 8 cm radius of curvature) was submerged in an acrylic tank filled with degassed deionized water. The tank was positioned below an MRI surface coil (14 cm diameter, constructed in-house) in a clinical 3T MRI scanner (Signa, General Electric Healthcare, Milwaukee, WI, USA) (Figure 2). The HIFU transducer was driven with waveforms produced by a function generator (model 395, Wavetek, San Diego, CA, USA), and amplified with an RF power amplifier (240L, E&I, Rochester, NY, USA); electric power was measured with a power meter (E4419B, Agilent) and a dual-directional coupler (C5948-10, Werlatone, Patterson, NY, USA). The acoustic output of the HIFU transducer

was calibrated using a force balance method (Maruvada *et al.*, 2007) to determine the acoustic power generated. Needle hydrophone measurements (diameter 0.2 mm, Precision Acoustics, Dorchester, UK) were acquired at acoustic powers < 2W (non-damaging to the hydrophone) and these measurements were used to extrapolate the peak negative pressures for the powers reported in the study. The location of the HIFU focus relative to the MRI coordinates was determined by heating a silicone gel phantom with a 10 W, continuous-wave ultrasound signal for 30 seconds. T₁-weighted MR images were acquired during sonication and the location of greatest change in MR signal intensity was identified as the HIFU focus. Each animal was then positioned in the MRI scanner with the tumor-bearing thigh immersed into the water at the center of the MR coil. T₂-weighted MR images (fast spin-echo sequence, TR = 4500 ms, TE = 84.9 ms, 12 cm × 12 cm image, 256 × 256 pixel resolution, slice thickness = 2 mm) were acquired before and after each sonication in order to guide the placement of the transducer focus within the tumor.

Two hours after injection, tumors were insonified with a 100-cycle pulse at an acoustic power of 90 W (peak negative pressure of 15.5 MPa) to vaporize accumulated PSNE followed by one million cycles at an acoustic power of 3.0, 4.1, or 5.2 W (peak negative pressures of 2.8 MPa, 3.3 MPa, and 3.7 MPa, respectively) for heating. This pulsing scheme was developed in prior studies using PSNE-loaded hydrogel phantoms (Zhang and Porter, 2010, Zhang *et al.*, 2013). The acoustic output parameters were selected to be lower than the intensities used by Sokka, et al., (2003) for generating bubbles directly in tissue without exogenous nuclei, and higher than the intensities used by Zhang, et al. (2011) to vaporize micron-sized perfluorocarbon droplets. During preliminary *in vitro* studies, we noted that the submicron droplets required higher acoustic pressures for vaporization compared to micron-sized droplets. The two hour delay was based on results from an earlier study indicating that PSNE accumulation in intramuscular rabbit VX2 tumors plateaued two hours after injection (Kopechek *et al.*, 2013). The combination of vaporization and heating bursts was repeated every 670 ms for 30 seconds. MR thermometry based on the temperature sensitivity of the water proton resonant frequency (PRF) was employed to monitor HIFU-induced heating as described previously (McDannold *et al.*, 1998). Image slices were acquired with a fast spoiled gradient-echo MR sequence using a TR of 27 ms, TE of 13.5 ms, dimensions of 8 cm × 8 cm, slice thickness of 2 mm, and a resolution of 256 × 256 pixels. Forty temperature maps were acquired at 3.4 s intervals at a single slice in the focal plane of the HIFU transducer. Temperature changes were estimated using phase contrast imaging (Ishihara *et al.*, 1995) and assumed a temperature sensitivity of the water PRF −0.01 ppm/°C (Hindman, 1966). Since the thermal dose has shown better correlation with tissue damage than temperature (McDannold and Jolesz, 2000; Sapareto and Dewey, 1984), thermal dose maps were computed from the acquired temperature maps using Equation 1 as previously described (McDannold *et al.*, 2000):

$$CEM_{43} = \sum_{t=0}^{t=final} R^{(43-T)} \Delta t, \quad (1)$$

where CEM_{43} represents the thermal dose in cumulative equivalent minutes at 43 °C, R is a constant value of 0.5 for temperatures above 43 °C or 0.25 for temperatures below 43 °C,

and T represents the mean temperature ($^{\circ}\text{C}$) measured during time t (minutes). The mean temperature and thermal dose was computed for a 0.83-mm diameter region of interest (chosen due to the -6 dB beamwidth) centered at the transducer focus. All four sonication conditions were tested in each tumor at four separate locations spaced at least 0.5 cm apart to avoid sonicating overlapping areas. Each sonication condition was tested in three separate tumors.

2.4 Cavitation Detection and Signal Analysis

Acoustic emissions from inertial cavitation were acquired using a custom-built, MR-compatible, 650-kHz ring hydrophone (Sokka *et al.*, 2003). The signals were digitized at a sampling rate of 10 MHz using a 12-bit digitizer card (PXI-5124, National Instruments, Austin, Texas, USA) and post-processed with Matlab (Mathworks, Natick, MA, USA). An MR trigger signal was acquired simultaneously in order to gate out signal interference from the MR pulses. As shown in Figure 3B, a 150- μs time-gate and a 1-ms time-gate was applied to window the broadband emissions detected during the vaporization (100 cycles) and cavitation (million cycles) bursts, respectively. The frequency spectrum of each gated signal was computed and the broadband emission dose was calculated by averaging the signal amplitude between 600 and 700 kHz (Figure 3C). Twenty hydrophone signals were acquired during the thirty-second HIFU exposures and the mean broadband emission dose was calculated for the vaporization and cavitation bursts, respectively.

2.6 Histology

Rabbits were sacrificed immediately following HIFU treatment and the tumors were removed and embedded in paraffin. Paraffin-embedded tumors were sliced and stained with hematoxylin and eosin (H&E) for examination in light microscopy. Images were acquired using objectives with magnifications between 0.625X and 20X.

2.7 Statistical Analysis

A 2-tailed Student's t -test was used to perform statistical analysis on the results (OpenEPI, Emory University, Atlanta, GA, USA). A p -value below 0.05 was the threshold used to determine statistical significance.

3. Results

3.1 PSNE Vaporization and Cavitation

Broadband emissions acquired by the ring hydrophone signals were processed to evaluate PSNE vaporization and cavitation. The broadband emissions detected during the vaporization burst before and two hours after PSNE injection is plotted in Figure 4A. The amplitude of broadband emissions during the 100-cycle, 90-W vaporization burst was significantly higher after PSNE injection, indicating that the droplets were vaporized and microbubbles formed. The broadband emissions detected during the million-cycle sonication before and two hours after PSNE injection is plotted in Figure 4B. The amplitude of the cavitation dose was significantly greater at 5.2 W after PSNE injection ($p < 0.01$), indicating that vaporized PSNE nucleated inertial cavitation. The broadband emission dose after PSNE vaporization was also compared at three different acoustic powers (3.0 W, 4.1 W, and 5.2

W), but there was no significant difference ($p > 0.05$). Representative plots of cavitation dose as a function of time are shown in Figure 4C at three different acoustic powers (3.0 W, 4.1 W, and 5.2 W), demonstrating that cavitation activity decreased during the 30-second HIFU treatment as PSNE were vaporized.

3.2 PSNE-enhanced Heating and Ablation in Tumors

The temperature and thermal dose maps for 5.2-W HIFU exposures before and two hours after PSNE injection are overlaid on T_2 -weighted MR images and shown in Figure 5. In the absence of vaporized PSNE (*i.e.* before PSNE injection), the average temperature in the implanted tumors increased by 12.2 ± 2.7 °C, whereas the average temperature measured after PSNE vaporization increased by 17.8 ± 2.1 °C ($p < 0.05$). Furthermore, in the case of 5.2-W exposures, it appears that PSNE vaporization and subsequent inertial cavitation increased the area of HIFU-induced heating that was detected by MR thermometry. The area where the thermal dose exceeded 240 cumulative equivalent minutes at 43 °C was approximately 4.9 mm² after PSNE injection compared to 1.3 mm² before PSNE was injected ($p < 0.05$). Thus, the thermal maps show that PSNE vaporization can increase the amount of heat generated as well as increase the area of heated tumor.

The mean temperature, thermal dose, and estimated lesion area are plotted in Figure 6 before and two hours after PSNE injection as a function of time and acoustic power. The mean temperature was 6 °C higher after PSNE vaporization compared to without vaporization, an increase of $46 \pm 22\%$. This resulted in a thermal dose increase of 3650 cumulative equivalent minutes at 43 °C. Although this difference may not appear to be large, it resulted in a significant increase in the thermal dose (3650 cumulative equivalent minutes at 43 °C). The thermal dose has shown better correlation with tissue damage than temperature (McDannold and Jolesz, 2000; Sapareto and Dewey, 1984). A thermal dose of 240 cumulative equivalent minutes at 43 °C is generally used as the threshold of coagulative necrosis (Chung *et al.*, 1999; McDannold *et al.*, 1998). Thus, the estimated lesion area was determined by computing the area where the thermal dose exceeded 240 cumulative equivalent minutes at 43 °C. Two hours after PSNE injection the temperature, thermal dose, and estimated lesion area at an acoustic power of 5.2 W were significantly higher than before PSNE injection, suggesting that PSNE vaporization enhanced HIFU-induced heating. The temperature, thermal dose, and estimated lesion area at 5.2 W before PSNE injection was similar to the results at acoustic powers of 4.1 W and 3.0 W after PSNE injection. This indicates that, for the range of acoustic powers tested in this study, the presence of inertial cavitation nucleated by vaporized PSNE increased the efficiency of heating above a critical acoustic power.

To evaluate the correlation between cavitation dose and heating in the tumors, the mean thermal dose after 30 seconds of sonication is plotted as a function of the average cavitation dose in Figure 7 for measurements acquired two hours after PSNE injection. Up to a critical value in the relative cavitation dose ($\sim 7.5 \times 10^2$), the measured thermal dose was negligible. Beyond the critical value, the measured thermal dose increased dramatically. Increasing the relative cavitation dose by almost a factor of two led to an increase in thermal dose by three

orders of magnitude. Thus, beyond a critical level of activity, cavitation significantly enhanced HIFU-mediated heating for the tested frequency and duration of insonation.

3.3 Tumor Histology

Representative histological slices are shown in Figure 8 from tumors exposed to 5.2 W HIFU before and after PSNE injection. Viable tumor cells stained purple, non-viable tumor cells stained pink, and red blood cells stained red. Before PSNE injection, a very small region of non-viable cells was observed after HIFU exposure and no evidence of thermal or vascular damage was visible. After PSNE injection and HIFU exposure, however, evidence of thermal and vascular damage within the tumor was observed, as indicated by the empty regions (likely from thermal necrosis) and the red spots (due to vascular damage).

4. Discussion

In vivo studies have shown previously that injected ultrasound contrast agents can reduce the power required for HIFU-mediated ablation (McDannold *et al.*, 2006) as well as increase the volume of ablated tissue (Chung *et al.*, 2012; He *et al.*, 2011; Kaneko *et al.*, 2005; Luo *et al.*, 2009; McDannold *et al.*, 2006; Tung *et al.*, 2006; Umemura *et al.*, 2005). However, circulating UCA can enhance HIFU-mediated heating at multiple points along the beam path, leading to unwanted thermal damage to tissue proximal to the transducer focus (McDannold *et al.*, 2006). Instead of injecting UCA, microbubbles can be generated at the focal spot, which would allow for better control temporally and spatially of bubble-enhanced HIFU (Sokka *et al.*, 2003). Previously, we reported that PSNE can accumulate within solid tumors, and be vaporized by MR-guided HIFU, thus confining bubble-enhanced HIFU to the focal spot (Kopechek *et al.*, 2013). In the current study, we monitored for inertial cavitation after PSNE vaporization within solid tumors and examined the relationship between inertial cavitation activity and HIFU-mediated heating and ablation.

Inertial cavitation was detected for all exposure power levels after PSNE vaporization, and a relative cavitation dose was calculated by summing the power contained in acoustic emissions between 0.6–0.7 MHz. We noted a significant increase in relative cavitation dose as the acoustic power of the long tone-burst was increased from 4.1 W to 5.2 W. It may be possible that beyond a critical acoustic power level, cavitating bubbles may vaporize additional PSNE, which will result in a noticeable enhancement in cavitation activity. This phenomenon has been demonstrated previously in an *in vitro* study in which cavitating ultrasound contrast agents reduced the pressure threshold for PFC droplet vaporization (Lo *et al.*, 2007). Interestingly, the difference in peak negative acoustic pressure from 4.1 W to 5.2 W (3.3 to 3.7 MPa) is similar to the threshold reported by Lo, et al., for this effect (3.8 MPa for 20 ms pulses at 1.44 MHz). Although the droplets used in their study were significantly larger (on the order of several microns in diameter compared to 169 nm in our study), it is possible that a similar effect occurred in the VX2 tumors. In addition to the increase in relative cavitation dose, we measured a significant increase in the peak temperature and the thermal dose as the acoustic power of the long tone-burst was increased from 4.1 W to 5.2 W. This finding suggests there is a minimal level of inertial cavitation activity required to significantly increase heat deposition in VX2 tumors for the insonation frequency and exposure duration used in the study. As shown in Fig. 7a relative cavitation

dose below a critical value resulted in negligible detected heat deposition in VX2 tumors. It is very likely that the heat generated by tissue absorption of energy radiated by cavitating bubbles diffused into neighboring tissue and was carried away by blood flowing through the tumor. Consequently, there was no measureable change in temperature during HIFU exposure. Beyond a critical value, there was a strong correlation between mean thermal dose and relative cavitation dose. These results are consistent with prior work in tissue-mimicking hydrogels *in vitro*, which demonstrated that cavitation enhances HIFU-mediated heating (Chen *et al.*, 2012; Farny *et al.*, 2010; Kyriakou *et al.*, 2011). In particular, Zhang, et al. (2013) showed that PSNE in tissue-mimicking hydrogels could be vaporized to nucleate cavitation in order to significantly enhance HIFU-mediated heating (Zhang *et al.*, 2013). However, in this study it was found that inertial cavitation nucleated by vaporized PSNE persisted longer (~15 s) in the solid tumors than what was reported from experiments in hydrogels (<1s) (Zhang *et al.*, 2013). This is an important finding given that inertial cavitation is a critical factor in bubble-enhanced ultrasound-mediated heating. The difference in inertial cavitation persistence may be due to differences in PSNE concentration as well as the number of PSNE that are vaporized. Further studies are warranted to evaluate the relationship between inertial cavitation activity, PSNE concentration, and acoustic parameters.

In VX2 tumors where cavitation was detected, we measured a significant increase in the area of heat deposition, possibly due to scatter of incident acoustic waves as well as radiation of acoustic energy into tissue by cavitating bubbles. While there was an increase in the heated area, inertial cavitation and enhanced HIFU-mediated heating remained confined to the transducer focal zone. Thus, the use of PSNE that has accumulated within tumors as cavitation nuclei instead of UCA reduced the risk of unwanted thermal damage to intervening tissue.

During the 5.2W sonications, the apparent temperature rise increased by 46% when the sonications were enhanced by vaporized PSNE. However, care must be taken in interpreting these results. Additional work is needed to verify that MR temperature imaging is accurate after vaporization of the PSNE. The generation of microbubbles might produce changes in magnetic susceptibility large enough to induce error in the temperature maps. This error might be largest in the center of the heated region, where presumably the temperature and thermal dose will be far above the threshold for tissue coagulation. Near the edge of the heated zone, where the temperature rise may be predominately the result of thermal conduction, the number of bubbles should be small, and presumably any errors in the temperature imaging could be relatively minor. If that is the case, the extent of the thermal lesion could still be predicted by the isodose contours.

It is possible that the peak temperature rise induced by sonication after PSNE injection could have been underestimated in this study. Previous work with cavitation-enhanced heating and MR temperature imaging has observed that the maximal point of heating can migrate towards the transducer due to reflections from a bubble cloud (Elbes *et al.*, 2013; Sokka *et al.*, 2003). Here, the thermometry was obtained in the focal plane (i.e., imaging oriented perpendicular to the direction of ultrasound beam propagation), and the heated region could have migrated out of the imaging plane during the 30 s sonications without our knowledge.

To examine this issue, a temperature map was acquired in the sagittal plane and negligible migration of the heated region was observed (Figure 9). It is possible that the *in situ* concentration of vaporized droplets may be lower than the microbubble concentrations in the referenced studies and thus may lead to less migration of the heated region. This topic will be explored further in future studies.

An illustration of the anticipated procedure for clinical PSNE-enhanced HIFU ablation procedures is shown in Figure 10. After intravenous injection, the PSNE droplets accumulate in the tumor through the EPR effect. Short ultrasound pulses are used to vaporize the droplets *in situ* within the tumor, forming microbubbles that serve as cavitation nuclei in order to enhance HIFU-mediated heating and tumor ablation. Liquid perfluorocarbons, including perfluoropentane, have been safely used in human studies without minimal adverse events (Lowe, 2001). Clinical studies primarily focused on using liquid perfluorocarbons as artificial oxygen carriers and ultrasound contrast agents (Diaz-Lopez *et al.*, 2010). In addition, perfluorocarbon gas is currently used clinically in ultrasound contrast agents, including formulations with FDA approval for left ventricular opacification (Kaul, 2008).

The goal of this study was to evaluate the effect of vaporized PSNE on HIFU-mediated heating and lesion formation in tumors. With the advent of cavitation mapping with transducer arrays (Gyongy and Coussios, 2010; Haworth *et al.*, 2011; Salgaonkar *et al.*, 2009), it will be possible to examine the relationship between the heating zone area with the size and geometry of the cavitation field nucleated by PSNE vaporization. Potentially, this would allow for feedback control of bubble-enhanced HIFU ablation and increase the predictability of cavitation-enhanced HIFU tumor ablation. Entire tumor ablation was not attempted in this study but is a topic to be explored in future studies. However, the results indicate that PSNE will likely reduce the time and/or acoustic intensities required to ablate tumors *in vivo*.

5. Conclusions

The results of this study demonstrated that phase-shift nanoemulsions were vaporized in rabbit VX2 tumors and nucleated cavitation activity which was correlated with enhanced HIFU-mediated heating and lesion formation. This suggests that PSNE could potentially reduce the time and/or acoustic intensities required for high intensity focused ultrasound-mediated thermal ablation of inoperable solid tumors, thereby improving the feasibility and efficacy of the treatment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.
Schematic of droplet in PSNE with liquid perfluorocarbon core and lipid shell containing polyethylene glycol chains.

Figure 2.

Diagram of the setup for MR-guided HIFU experiments. The animal was placed in a 3T magnet and the tumor oriented above a 1.5 MHz HIFU transducer. A custom-built ring hydrophone was placed around the HIFU transducer to detect cavitation emissions.

Figure 3.

A) Diagram of the input pulse containing a short, high-amplitude pulse to vaporize PSNE followed by a long pulse to sustain cavitation activity. B) Time-domain signals from tumors with and without PSNE (shaded regions indicate vaporization pulse, left, and cavitation pulse, right), C) Corresponding frequency spectra (shaded regions indicate frequency bands used for analysis).

Figure 4.

(A) Broadband emissions dose from the vaporization pulse before and after PSNE injection, indicating that PSNE were vaporized into microbubbles during the vaporization pulse. (B) Cavitation dose from the million-cycle pulse before and after PSNE injection at different acoustic powers. The cavitation dose was significantly higher at 5.2 W after PSNE injection compared to before PSNE injection ($p < 0.01$), but there was no difference between acoustic powers after PSNE vaporization ($p > 0.05$). Bars represent the mean $\pm$ standard deviation from measurements with three animals. (C) Representative plots of cavitation dose as a function of time during the 30-second HIFU treatment.

Figure 5. Temperature and thermal dose maps in rabbit tumors after 30 seconds of sonication at 5.2 W before and two hours after PSNE injection, overlaid on T₂-weighted MR images. The temperature and thermal dose were higher after PSNE injection. The location of the HIFU focus is indicated by the white circle on each image. A video loop of the temperature and thermal dose maps as a function of time is available in the online version.

Figure 6.

(A) Mean temperature, (B) mean thermal dose, and (C) estimated area of ablation before and after PSNE injection as a function of time and acoustic power. The estimated area of ablation was computed from the area where the thermal dose exceeded 240 CEM₄₃. The green box indicates the time when tumors were sonicated. These results indicate that vaporized PSNE significantly enhanced the rate of heating and ablation in the tumors. Data points represent the mean $\pm$ standard deviation from measurements with three animals.

Figure 7.

Correlation of mean thermal dose after 30 seconds of sonication with the average cavitation dose two hours after PSNE injection. The blue line indicates the correlation for all points on the plot, whereas the red line indicates the correlation for points above a relative cavitation dose value of 6×10^2 . The results indicate that above a critical value of cavitation activity nucleated by vaporized PSNE, HIFU-mediated heating was significantly enhanced in tumors.

Figure 8. Representative histological slices from tumors exposed to 5.2 W HIFU (A) without PSNE injection and (B) after PSNE injection. Evidence of thermal and vascular damage was observed from tumors exposed to 5.2 W HIFU after PSNE injection. Scale bar represents 100 μm .

Figure 9. Thermal dose map acquired in the sagittal plane of a tumor containing PSNE and sonicated at an acoustic power of 5.2 W. Migration of the heated region toward the transducer was minimal.

Figure 10.

Illustration of anticipated process for clinical PSNE-enhanced HIFU ablation procedures. (1) PSNE is injected intravenously and accumulates in tumor through EPR effect. (2) Short, high-amplitude HIFU pulses vaporize PSNE into microbubbles in tumor. (3) Microbubbles formed by vaporized PSNE enhance HIFU-mediated heating and ablation of tumor.