In vivo hyperthermia effect induced by high-intensity pulsed ultrasound*

Cui Wei-Cheng(崔炜程)[a], Tu Juan(屠 娟)[a]†, Hwang Joo-Ha[b], Li Qian (李 倩)[a], Fan Ting-Bo(范庭波)[a], Zhang Dong(章 东)[a], Chen Jing-Hai(陈静海)[c], and Chen Wei-Zhong(陈伟中)[a]

[a] *Key Laboratory of Modern Acoustics, MOE, and Institute of Acoustics, Nanjing University, Nanjing* 210093, *China*

[b] *Center for Industrial and Medical Ultrasound, Applied Physics Laboratory, University of Washington, Seattle,* WA 98105, *USA*

[c] *Department of Cardiology of Children's Hospital Boston, Harvard Medical School, Boston,* MA 02115, *USA*

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Hyperthermia effects (39–44 °C) induced by pulsed high-intensity focused ultrasound (HIFU) have been regarded as a promising therapeutic tool for boosting immune responses or enhancing drug delivery into a solid tumor. However, previous studies also reported that the cell death occurs when cells are maintained at 43 °C for more than 20 minutes. The aim of this study is to investigate thermal responses inside *in vivo* rabbit auricular veins exposed to pulsed HIFU (1.17 MHz, 5300 W/cm^2, with relatively low-duty ratios (0.2%–4.3%). The results show that: (1) with constant pulse repetition frequency (PRF) (e.g., 1 Hz), the thermal responses inside the vessel will increase with the increasing duty ratio; (2) a temperature elevation to 43 °C can be identified at the duty ratio of 4.3%; (3) with constant duty ratios, the change of PRF will not significantly affect the temperature measurement in the vessel; (4) as the duty ratios lower than 4.3%, the presence of microbubbles will not significantly enhance the thermal responses in the vessel, but will facilitate HIFU-induced inertial cavitation events.

Keywords: hyperthermia effects, high-intensity focused ultrasound, duty ratio, ultrasound contrast agents

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1. Introduction

Ultrasound, as a physical power, has been introduced to many fields including industry, chemistry, and medicine. As ultrasound waves propagate through tissue, a portion of acoustic energy is absorbed and converted to heat, which increases the temperature of the tissue.[1−5] High-intensity focused ultrasound (HIFU), with its ability to accurately target and non-invasively deposit high energy deeply inside the body, has draw much attention in medical therapy for decades.[6,7] HIFU can be employed in two ways: continuous and pulsed exposures. Continuous HIFU can rapidly increase temperature to higher than 70 °C at the beam focus, which could be used clinically to treat both malignant and benign tumors[8−11] and ablate the targeted tissue within just seconds.[12] However, the rapid temperature elevation induced by continuous HIFU is hard to monitor and control in real time, which could result in undesirable thermal injury to surrounding normal tissues.[13] Compared with continuous HIFU, pulsed HIFU exposures not only can generate clinically desired non-thermal effects (e.g., inertial cavitation and radiation force) to facilitate the production of ultrasound-mediated drug, gene delivery,[14−16] and thrombolysis,[17,18] but also can achieve a relatively stable temperature within a hyperthermia range of 39–44 °C.[7] Many researchers have reported that the hyperthermia effect can increase vascular permeability[19] and oxygenation,[20] and also activate the spontaneous anti-tumor immune response.[7,21,22] Therefore, pulsed HIFU-induced hyperthermia has been regarded as a promising safer technique for cancer treatment. In the 1980s, ultrasound-induced hyperthermia was used

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†Corresponding author. E-mail: juantu@nju.edu.cn

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to treat superficial human cancerous nodules.[23] Furthermore, hyperthermia-activated gene expression has been achieved *in vivo* by using focused ultrasound guided by magnetic resonance imaging (MRI).[24] More recently, pulsed HIFU-induced hyperthermia was also employed with the administration of heat-sensitive liposomes to enhance the drug delivery into solid tumors.[25,26]

Despite the growing interest in pulsed HIFU-induced hyperthermia, the majority of researchers just empirically selected HIFU parameters for their different applications, and no systemic study has been performed *in vivo* to investigate how the hyperthermia temperature is affected by the duty ratio (DR) of the pulsed HIFU. The aim of the current work is to examine the temperature elevation resulting from low duty-ratio ultrasound exposures with high spatial peak pulse average intensities (I_{SPPA}) in the marginal veins of a rabbit ear *in vivo*. All the HIFU exposures were performed at the same driving frequency of 1.17 MHz and intensity of 5300 W/cm^2, but at different duty ratios. The temperature elevation in the vein of a rabbit ear with/without adding anultrasound contrast agent (UCA) microbubbles were measured with a thermocouple, and inertial cavitation (IC) broadband noise signals were monitored by means of passive cavitation detection (PCD) technology. Three hypotheses are made as follows: (1) the thermal response of the tissue to low duty-ratio and high-I_{SPPA} ultrasound waves will be enhanced with the increase of the duty ratio, (2) a duty ratio corresponding to a temperature rise to 43 °C can be identified, which is sufficient to generate hyperthermia bioeffects, and (3) the presence of circulating UCA microbubbles may not significantly change the thermal response of the tissue to pulsed HIFU with a low duty ratio. The results in this paper will help us better understand the mechanism of the ultrasound-induced hyperthermia effect, which should be beneficial for clinical pulsed HIFU-assisted treatment of cancer.

2. Materials and methods

2.1. Ultrasound contrast agents

The UCA commonly used in experiments is Optison® (Princeton, New Jersey: Amersham Health). The current maximum FDA-approved dose of Optison® is 8.7 ml for diagnostic purposes, which corresponds to a void fraction of about 10^{-5}. Therefore, in the current treatments with UCA microbubbles, 0.5 ml Optison® is injected immediately prior to the ultrasound exposure (corresponding to an intravascular void fraction of about 10^{-5}). The interval between UCA injections is 10 minutes, which was determined by the clearance time of Optison® measured by Tu *et. al.*[27] using the same rabbit ear vein system.

2.2. Ultrasound exposure setup

Figure 1 shows the diagram of the experimental setup. The ultrasound transducer is comprised of an air-backed, 34.9-mm (diameter) APC880 disk (Mackeyville, Pennsylvania: APC International Ltd.) attached to a custom-built aluminum focusing lens with a radius of curvature of 5 cm. The driving electronics consists of a waveform generator 33120A (Palo Alto, CA, USA: Agilent Technologies) and an RF power amplifier AP-400B (Rochester, NY, USA: ENI). To provide acoustic coupling, a custom-built truncated cone is attached to a transducer and filled with degassed water. The tip of the cone is covered with a polyurethane membrane with an O-ring seal. The cone is manufactured to ensure that the focus of the transducer's output is exactly at the tip of the cone. The transducer is then mounted to a water tank.

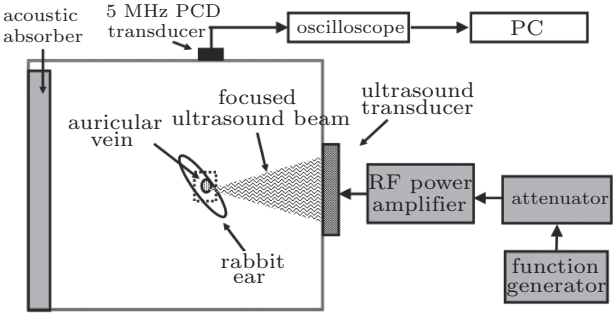


Fig. 1. Schematic diagram of the experimental system.

Prior to performing experiments, the transducer was calibrated in the tank filled with degassed water. A membrane hydrophone MHA 200A (Seattle, WA, USA: NTR Systems Inc.) was mounted to a precise three-dimensional (3D) motion stage and used to determine the spatial characteristics of the transducer beams. Also, the relationship between the input voltage and output acoustic pressure at transducer's focus was determined by using the membrane hydrophone. The transducer was operated at its resonance frequency of 1.17 MHz. The maximum achievable acoustic pressure amplitudes with this system are 27 MPa peak positive and 9 MPa peak negative.

The I_{SPPA} was calculated directly from the hydrophone measurement by initially determining the instantaneous intensity (i) via the following equation: $i = p^2/\rho c$, where p is the instantaneous acoustic pressure, the blood density $\rho = 1057$ kg/m^3, and the acoustic velocity in blood $c = 1575$ m/s. The I_{SPPA} was then determined by integrating the instantaneous intensity over 20 cycles, i.e., $I_{\mathrm{SPPA}} = (nT)^{-1} \int_{nT} i\mathrm{d}t$, where $n = 20$, the measurement sampling rate $\mathrm{d}t = 40$ ns/pt, and $T = 8.54 \times 10^{-7}$ s (the period of one cycle at 1.17 MHz). Finally, it is derived based on these calculations that $I_{\mathrm{SPPA}} = 5300$ W/cm^2.

2.3. Animal treatment protocol

Five New Zealand white rabbits weighing 4–5 kg each were used for these acute experiments, which were carried out according to the NIH guidelines. Following initial sedation with a subcutaneous injection of a cocktail of acetylpromazine (1.0 mg/kg) and ketamine (22 mg/kg), the auricular surfaces were shaved and depilated to facilitate the ultrasound coupling. A 21 gauge catheter was inserted into the proximal auricular vein of one ear through intravenous access. The animals were anesthetized with an intravenous injection mixture of ketamine (35–40 mg/kg) and xylazine (5 mg/kg) and placed in a lateral decubitus position in preparation for treatment. Rabbits remained anesthetized throughout the entire experimental process. At the end of the experiment, the rabbits were euthanized with an overdosed intravenous injection of ketamine/xylazine cocktail.

2.4. Thermocouple measurements

After achieving anesthesia, the rabbit was placed on a custom-built platform mounted to a precise 3D motion stage. The water tank was filled with degassed water and remained at 36–38 °C by using a circulating water heater (West Chester, PA: VWR International). An acoustic absorber was placed along the distal tank wall to minimize reflections from the tank wall and thus reduce standing wave formation. The rabbit ear vessel model was selected because the vessel is close to the surface of the ear, allowing for accurate HIFU targeting and good signal detection, and the auricular vein is of sufficient dimension (a diameter of about 1–2 mm) to allow the placement of thermocouple. During the experiments, the rabbit ear is hold in position on a custom-built rigid holder (with a 10×6 cm^2 acoustic window) such that the targeted region of the vessel can be placed reliably at the focus of the ultrasound beam. This system would provide accurate, reproducible positioning of a targeted segment of the auricular vein into the focal region of the transducer, which also correlated with the focal region of the PCD transducer.

Temperature measurements were undertaken *in vivo* by using an E-type thermocouple (Stanford, CT, USA: Omega Engineering Inc.) with an outer diameter of 0.01 inch (about 250 μm). Figure 2 shows the positioning of the thermocouples (position 1) within the rabbit ear vein. Temperature data were acquired by using a data acquisition break-out box controlled by the LabView software. The sampling rate of the data acquisition system was 30 ms^{-1}. The temperature response time for the thermocouple, according to data sheets provided by the manufacturer, is 0.3 s. Therefore, the temperature response time of the thermocouple was the limiting factor in acquiring temperature measurements for pulses that are shorter than 0.3 s. The measured thermal data were finally plotted and analyzed via Origin 7.5. The change in temperature (ΔT) was determined by taking the steady-state value of the measured temperature at 1 min and then subtracting it from the baseline temperature measurement obtained prior to the ultrasound exposure.

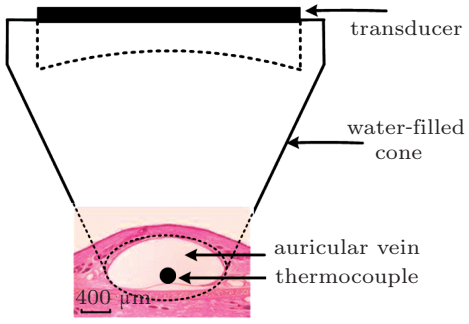


Fig. 2. (colour online) Schematic drawing of the placement of thermocouple. The rabbit auricular vein, whose mean diameter is about 1–2 mm, is close to the ear surface with a distance of about 200 μm.
The tip of the thermocouple with a diameter of about 250 μm (marked as the black dot) is placed within the lumen of the auricular vein against the distal wall of the vessel. A water-filled cone was used to accurately couple the acoustic energy into the vein. The focus of the transducer (dashed ellipse) is large enough to cover the whole auricular vein.

2.5. Broadband noise detection for the inertial cavitation activities

Since the pulsed HIFU signals were driven at a high negative pressure of 9 MPa, the IC activities

might be generated due to the HIFU exposures.[27] A 5-MHz "listening" transducer, whose focal volume overlapped with that of the source transducer, was used in our studies to detect broadband acoustic scattering and emission signals from the oscillating UCA microbubbles. These signals were sampled with the oscilloscope LC 334AM (Chestnut Ridge, NY, USA: Lecroy Co.) by using the sequence mode with a time resolution of 40 ns per point. A LabView program (Austin, TX, USA: National Instruments Co.) was built for data collection. All the post signal analysis programs were written in MatLab (Natick, MA, USA: Math Works).

3. Experimental series and results

3.1. Effects of variable duty ratio on temperature elevation

In this series of experiments, multiple vessel segments were treated along the rabbit auricular vein, with treatment segments separated by 1 cm. The same treatment protocol was applied to each individual rabbit. The experimental protocol consisted of a 10-s baseline measurement where no ultrasound exposure occurred, followed by a 60-s exposure of ultrasound (driving frequency: 1.17 MHz, pulse repetition frequency (PRF): 1 Hz, $I_{\mathrm{SPPA}} = 5300$ W/cm^2) with a pulse length of 2500, 5000, 10000, 25000, or 50000 cycles, corresponding to a duty ratio of 0.2%, 0.4%, 0.9%, 2.1%, or 4.3%, respectively. Then, each rabbit initially measured without any UCA injection was injected with the UCA. Preliminary studies demonstrate that, following the injection of 0.5-ml Optison®, there was no detectable IC activity above the baseline (i.e., without Optison®) after about 9 min. Therefore, a 10-min waiting time between two consecutive treatments was left to avoid the influence of the former injection. Five replicated experiments were conducted for each measurements in this series.

The changes in temperature measured inside the rabbit auricular vein exposed to ultrasound exposures with/without the UCA are plotted as a function of the acoustic duty ratio in Fig. 3. It can be found that the temperature increases with the increasing acoustic duty ratio. There is no significant difference in temperature measurements in vessels with or without the UCA. With a duty ratio of 4.3%, the temperature rise of about 5 °C was measured. Since the baseline temperature of the vessel was 37–38 °C, a temperature rise to 42–43 °C would be achieved with the pulsed HIFU exposure at a duty ratio of 4.3%, which should be sufficient to induce hyperthermia effects in the vessels.

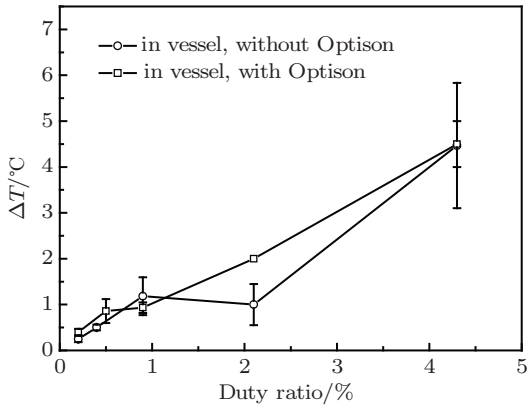


Fig. 3. Relationship between the duty ratio and the temperature increase measured by virtue of thermocouple placed with/without the UCA.

3.2. Effects of variable PRF on the change of temperature

In a periodic event, duty ratio refers to the ratio of the pulse duration to the total pulse period. In the present studies, the duty ratio can be determined by multiplying the pulse length by T and PRF. Since the source HIFU transducer was driven at a fixed frequency of 1.17 MHz and a pulse period of 8.54×10^{-7} s. Therefore, the duty ratio could be adjusted by changing either the pulse length or the PRF.

In this series of experiments, the effects of variable PRF on the temperature inside the vessels were also studied at fixed duty ratios. The experimental protocol also consisted of a 10-sec baseline measurement where no ultrasound exposure occurred, followed by a 60-sec ultrasound exposure (driving frequency: 1.17 MHz, $I_{\mathrm{SPPA}} = 5300$ W/cm^2) without the UCA injection. The PRFs studied in the experiments were 1, 10, 50, and 100 Hz, respectively. Correspondingly, the pulse length is adjusted to keep duty ratios constant. Five replicated experiments were conducted for each measurements in this series.

Figure 4 shows the change in temperature measured with varied PRF. It is observed that, as long as the duty ratios keep constant, the change of PRF does not make significant differences to the temperature measured inside the rabbit auricular vein.

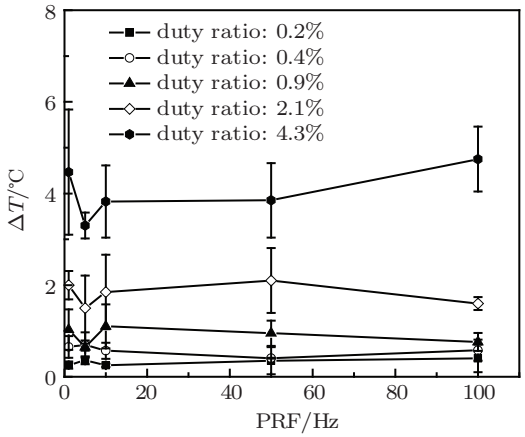


Fig. 4. Relationship between the PRF and the temperature measured via the thermocouple placed inside the rabbit auricular vein without the UCA.

3.3. Inertial cavitation-induced broadband noise detection based on PCD measurements

It is well known that the destruction of cavitation microbubbles are characterized by the emission of broadband noise signals, which can be sensitively detected by the PCD system. Therefore, except for the temperature measurements, the IC-induced broadband noise signals were also detected via a 5-MHz "listening" transducer. Figure 5 shows the fast Fourier transform (FFT) spectra of emitted signals detected in rabbit auricular veins exposed to the HIFU pulses with a duty ratio of 0.2%. As shown in Fig. 5(a), even for the employed source HIFU transducer operating at the lowest duty ratio (i.e., 0.2%), broadband harmonic components can still be observed apparently, which indicates the occurrence of the violent IC activities. It is also obviously observed in Fig. 5(b) that the FFT amplitudes of harmonics can be enhanced dramatically with the addition of the UCA microbubbles, which should correspond to the aggravation of the IC events.

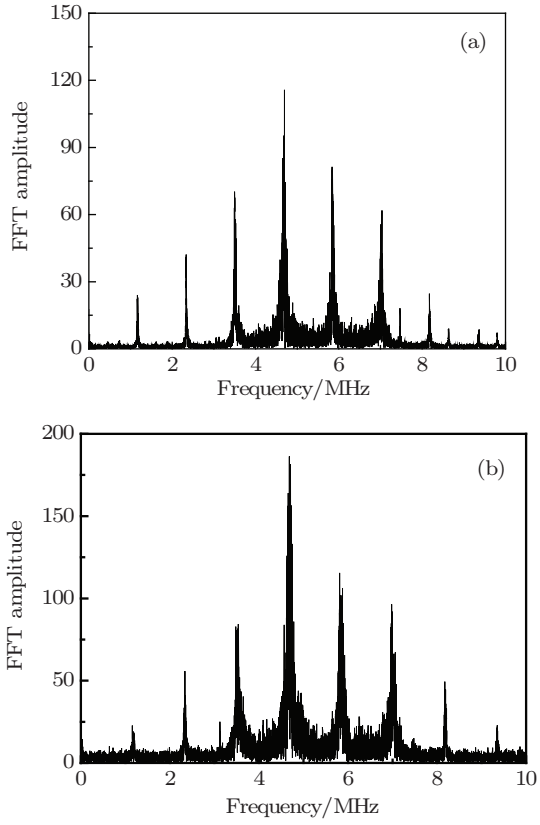


Fig. 5. IC-induced broadband noise detected in the rabbit auricular vein exposed to the HIFU pulses (a) without the UCA injection, and (b) with the UCA injection, based on the PCD technology. Driving frequency: 1.17 MHz, PRF: 1 Hz, $I_{\mathrm{SPPA}} = 5300$ W/cm^2, DR: 0.2%.

4. Discussion

In vivo experiments were performed to examine the temperature elevation resulting from low duty-ratio, high I_{SPPA} ultrasound exposures in the marginal vein of a rabbit ear *in vivo*, with the purpose of identifying ultrasound duty ratios that would induce significant hyperthermia effects while minimizing the probability of thermal injuries.

This study demonstrates the enhancement of thermal response of tissues to the pulsed HIFU exposures with increasing duty ratio. As expected, the experimental results demonstrate that lower duty ratios will result in smaller temperature rise, that is, essentially no obvious temperature elevation occurred at a duty ratio of 0.4% or less. A temperature elevation to 43 °C was identified when the 1.17-MHz HIFU transducer worked at a duty ratio of 4.3%, with PRF being 1 Hz and $I_{\mathrm{SPPA}} = 5300$ W/cm^2. A temperature elevation to 43 °C was specifically selected for this study because previous studies have demonstrated that the rate of cell death increases significantly for temperatures higher than 43 °C.[28,29] These studies were performed to determine the time–temperature relationship for heat-induced cell death. They reported that the exposure time at a certain temperature to kill 50% of the cells for cells exposed at 43 °C was longer than 20 min for all cell types being studied, and essentially no significant cell death occurred below 41 °C even under exposure of over an hour. Since a duty ratio of 4.3% was identified to raise the tissue temperature up to 43 °C, the experimental results indicate that a duty ratio less than 4% should be desirable for the current

HIFU system to generate effective hyperthermia effects without inducing significant thermal injuries in the tissue, as long as the exposure time is shorter than 20 min.

The experimental studies also demonstrates that, for a constant duty ratio, the change of PRF (or pulse length) will not make significant differences to the temperature measured inside the rabbit auricular vein. This result indicates that, for the pulsed HIFU system running at relatively low duty ratio, the ultrasound-induced hyperthermia effects should be more dominated by the accumulated acoustic energy deposition.

Deng *et. al.*[30] has reported that the acoustic pressure threshold for the IC events in whole blood is about 4.5 MPa. Since the current HIFU transducer is driven at a negative pressure of 9 MPa which is much higher than 4.5 MPa, the violent IC activities should be expected under the HIFU exposures. The results shown in Fig. 5 provide solid support for this hypothesis. It is also observed that the IC activities can be enhanced by adding extra cavitation nuclei (e.g., UCA microbubbles), which is consistent with pervious studies.[27] Although the microbubbles may be helpful in boosting IC activities, the present experimental results shows that the pulsed HIFU-induced hyperthermia temperature elevation will not be affected by the injection of UCAs at all. The possible explanation for this phenomenon might lie in the low duty ratio. Since the HIFU transducer works at pulsed mode, the heat deposition resulting from ultrasound exposures will dissipate gradually during the transducer's "off-time". When the transducer works at a low duty ratio, the sonicated tissue may have sufficient time to "cool down". Therefore, although HIFU-induced IC activities may be quite useful in other therapeutic applications (e.g., tumor ablation and gene/drug delivery), there may be other mechanisms (e.g., bio-heat transfer or micro-stream) inducing the hyperthermia effects that should be investigated further in our future work.

Another important point worthy of being noted here is that although no significant impact has been observed for the IC effect on the HIFU-induced hyperthermia temperature elevation, the occurrence of violent IC activities may still result in non-thermal-induced cell deaths, which may lower the safety threshold of the hyperthermia temperature and should be avoided in clinic.

5. Conclusions

In summary, this paper has demonstrated that, with the transducer used here and at its maximal output ($I_{\mathrm{SPPA}} = 5300$ W/cm^2 at the focus), a duty ratio of about 4% is sufficient to induce hyperthermia effects without producing any significant thermal injuries for exposures of less than 20 min in duration. Detailed results include: (1) the thermal response of tissues to pulsed HIFU with a given I_{SPPA} will increase with the increasing duty ratio; (2) a temperature elevation to 43 °C can be identified at the duty ratio of 4.3%; (3) for a constant duty ratio, the pulsed HIFU-induced hyperthermia effects will not be affected by the change of PRF (or pulse length); (4) the presence of microbubble ultrasound contrast agents will not significantly change the thermal responses in the vessel exposed to the HIFU pulses with low duty ratio, but will facilitate the HIFU-induced inertial cavitation events.

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