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Pulmonary Capillary Hemorrhage Induced by Fixed-Beam Pulsed Ultrasound

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Abstract

The induction of pulmonary capillary hemorrhage (PCH) by pulsed ultrasound was discovered 25 yr ago but early research utilized fixed-beam systems rather than actual diagnostic ultrasound machines. In this study, fixed-beam focused ultrasound exposures for 5 min at 1.5 MHz and 7.5 MHz were performed in rats for comparison to recent research with diagnostic ultrasound. One exposure condition at each frequency used 10 μ s pulses delivered at 25 ms intervals. Three conditions involved Gaussian modulation of the pulse amplitudes at 25 ms intervals to simulate diagnostic scanning: 7.5 MHz with 0.3 μ s and 1.5 μ s pulses at 100 μ s and 500 μ s pulse repetition periods, respectively, and 1.5 MHz with 1.7 μ s pulses at 500 μ s repetition periods. Four groups were tested for each condition to assess PCH areas at different exposure levels and to determine occurrence thresholds. The conditions with identical pulse timing showed smaller PCH areas for the smaller 7.5 MHz beam, but both had thresholds of 0.69–0.75 MPa in situ peak rarefactional pressure amplitude (PRPA). The Gaussian modulation conditions for 7.5 MHz with 0.3 μ s pulses and 1.5 MHz with 1.7 μ s pulses both had thresholds of 1.12–1.20 MPa PRPA, although the relatively long 1.5 μ s pulses at 7.5 MHz gave a threshold of 0.75 MPa. The fixed-beam pulsed ultrasound exposures produced lower thresholds than diagnostic ultrasound. There was no clear tendency for thresholds to increase with increasing ultrasonic frequency when pulse timing conditions were similar.

Keywords

Pulmonary ultrasound; comet tail artifact; bioeffects of ultrasound; ultrasound dosimetry; Mechanical Index

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Introduction

Pulsed ultrasound was reported to induce pulmonary capillary hemorrhage (PCH) in mice 25 years ago (Child et al. 1990). Subsequently, PCH has been studied by several different research groups and confirmed to occur with diagnostic ultrasound (AIUM, 2000; Church et al. 2008). This phenomenon is not only of basic scientific interest; it also appears to be the only clearly demonstrable bioeffect of diagnostic ultrasound reported to occur in mammals (in the absence of ultrasound contrast agents). Therefore, the PCH bioeffect could present a risk of injury and progression in these patients. Direct pulmonary examination by diagnostic ultrasound has become routine for diagnosis of patient conditions such as pulmonary edema and effusion, pulmonary embolism, atelectasis, diffuse parenchymal disease, adult and newborn respiratory distress syndrome, and lung cancer (Sartori and Tombesi, 2010; Dietrich et al. 2015). However, the risk of PCH induction remains poorly understood. The physical mechanisms and dosimetry have not been clearly defined. In addition, differences in methods, especially the use of fixed-beam pulsed modes (Church et al. 2008) versus recent diagnostic scanning research (Miller, 2012; Miller et al. 2015a) has produced a confusing range of results.

For a given set of conditions, ultrasonic PCH is not detectable at low values of exposure parameters, such as pressure amplitude or pulse-average intensity. Above a threshold, PCH increases in frequency of occurrence and in the area and volume of lung tissue involved. For near-threshold exposures the occurrence of PCH is notably indeterminate, with seemingly identical exposure of individual animals producing zero or some PCH. An improved understanding of the etiology of PCH has been sought by measuring the magnitude and threshold of the bioeffect for exposures with different conditions and parameters. However, the number of parameters and the possible ranges of their values, including the accurate determination of in situ values of exposure, complicate this process. Parameters include ultrasonic frequency, positive and negative pressure amplitudes, pulse or time average intensities, pulse duration, pulse repetition frequency, image frame rate, total on-time, and exposure duration for pulsed exposure. Because of the large number of parameters and their possible values, a complete compendium of thresholds and magnitudes of PCH in mammals is probably an unrealistic research aim. The variation in PCH observed with various limited parameter sets have been reviewed by AIUM (2000) and Church et al. (2008). Different species have been used in the research, including mice, rats, rabbits, pigs, monkeys and humans (AIUM, 2000; O'Brien et al. 2006; Church et al. 2008). In addition, biological conditions are important, including age (Dalecki et al. 1997; O'Brien et al. 2003) and the specific anesthesia techniques (Miller et al. 2014a; Miller et al. 2015b).

A key parameter for investigating the physical mechanism for PCH is the ultrasonic frequency. Seminal studies by Child et al. (1990) and Zachary et al. (2001) have indicated increasing thresholds of PCH for increasing frequency, based on exposures with laboratory research systems. An authoritative review by the American Institute of Ultrasound in Medicine (AIUM, 2000) analyzed thresholds at ultrasonic frequencies between 1 and 4 MHz. When these were expressed in terms of the pulse peak rarefactional pressure amplitudes (PRPA), adjusted for tissue attenuation, a fitted line had a threshold value of 0.63 MPa at 1 MHz and increased with frequency raised to the power of 0.54 (i. e. approximately

the square root of frequency, as for the Mechanical Index (MI), which is displayed on diagnostic ultrasound machines).

The relationship of PCH occurrence and magnitude to ultrasonic frequency may help to identify the mechanisms of PCH. Two prominent mechanisms for bioeffects of ultrasound are heating and cavitation. The thermal mechanism depends on temperature elevation, which has a complex dependence on frequency based on attenuation and absorption. However, heating is very low for pulsed ultrasound of low temporal average intensity. Tests for the involvement of heating have indicated that this mechanism is unlikely to explain ultrasound induced PCH (Hartman et al. 1992; Zachary et al. 2006). The cavitation mechanism would be expected to depend on ultrasonic frequency, possibly as described by the Mechanical Index, which depends on the square-root of frequency. However, tests for cavitation involvement in PCH did not support this hypothesis (Raeman, 1997; O'Brien et al. 2000, 2004). In addition, a study of PCH from this laboratory has indicated that, although the magnitude of PCH from diagnostic ultrasound decreased with increasing frequency, the threshold was approximately constant, rising only slightly in the range of 1.5 to 12 MHz (Miller et al. 2015a).

More definitive information on the etiology of PCH would be valuable to clarify the extensive data-base presently available and to assist in estimating risks. In particular, the relationship of information obtained in studies using laboratory pulsed ultrasound systems to those using diagnostic ultrasound machines is presently unclear. For example, studies using fixed pulsed beams of ultrasound have indicated that the thresholds for PCH increase for decreasing pulse durations at constant pulse repetition frequency (Child et al. 1990; O'Brien et al. 2003). For diagnostic scanning (Miller et al. 2015a), the pulse duration was less at higher frequencies, which improves axial resolution, and the pulse repetition frequency was also decreased for higher frequencies, which accommodates the reduced penetration. Therefore, in different studies, a reported frequency dependence of PCH thresholds may be related more to variations in pulse duration and pulse repetition frequency than to a specific dependence on ultrasonic frequency. In addition, the exposure to a point in tissue is quite different for fixed compared to scanned DUS beams, for which the pulse amplitudes vary with time as the pulsed beam scans past the point. The purpose of this present study of PCH was to clarify the relationship between exposures by fixed pulsed beams to scanned imaging beams.

Methods

Ultrasound

Ultrasound exposure was provided by a laboratory pulsed-ultrasound system with guidance by diagnostic ultrasound imaging, similar to systems described previously for ultrasound therapy (Miller et al. 2014b). The laboratory exposure system consisted of damped 1.5 or 7.5 MHz transducers (A3464 and A321S, Panametrics A3464, Olympus, Waltham, MA, USA), which were 1.9 cm in diameter and focused at 3.75 cm. The transducers were powered by an amplifier (A-500, Electronic Navigation Industries, Rochester NY) using a pulsed signal. A function generator (model 3314A, Hewlett Packard Co., Palo Alto CA) produced a continuous pulse train of a specific duration (number of whole cycles) and pulse

interval. For simulating a scanned-beam exposure, this pulse train was modulated to produce pulse-bursts of increasing, then decreasing pulse amplitudes (Miller et al. 2007). A repetitive Gaussian signal from an arbitrary waveform generator (model 33220A, Agilent Technologies, Loveland CO) was applied to the amplitude modulation input of the function generator.

The fixed-focus beam was aimed with the aid of a 10 MHz diagnostic ultrasound image (S10 probe, GE Vivid 7 Dimension, General Electric Corp., Cincinnati OH, USA) operated with 5 cm focal depth, as previously described (Miller et al. 2014b). A power setting of -20 dB (MI=0.1) was used to avoid potential lung injury during the B mode scanning for the aiming procedure. The imaging probe and exposure transducer were clamped together at a 37 degree angle so that the position of the exposure transducer focus was located in the image. For aiming, a bright lung surface was obtained in the image, avoiding the sternum and ribs on the right side of a rat, at a position with the lung surface at 2.25 cm depth at the edge of the sector scan. For exposure, the probe and transducer assembly was repositioned to locate the exposure point on the lung surface at the center of the image at a 2.75 cm image depth and 3.75 cm exposure depth, as shown in Fig. 1. This maneuver aligned the fixed transducer beam along the same line as for the image during aiming, and ensured that the transducer beam was directed between ribs, avoiding the sternum.

Exposures were performed under the different pulsing conditions listed in Table 1. All exposures were 5 min in duration. Simple pulse trains with 10 μ s duration pulses spaced 25 ms apart (40 Hz pulse repetition frequency) were created at 1.5 MHz (condition A) and 7.5 MHz (condition B). These conditions were intended to compare the two frequencies using identical pulse-timing parameters (except with different numbers of cycles). This strategy was similar to that of Child et al. (1990), for which 1.2 MHz and 3.7 MHz focused ultrasound was used to expose mice for 3 min to 10 μ s pulses with 10 ms pulse interval. The simulated scanning with Gaussian modulation was set with an equivalent frame interval of 25 ms (40 frames per s) for condition C at 1.5 MHz and also for conditions D and E at 7.5 MHz. The Gaussian modulation had a full width at half maximum of 1.9 ms. Conditions C and D were intended to compare the simulated scanning with the different frequencies, but the same timing parameters. Condition E was intended to simulate the previous B mode scanning method at 7.6 MHz (Miller, 2012), which had 0.3 μ s pulses, 100 μ s pulse interval and 39 fps. The simple pulse modes and simulated scan modes had the same repetition period (25 ms) but were not matched for parameters such as on-time. The pulse durations for conditions C and E were taken from the B mode exposures of Miller et al. (2015a), while the pulse duration for condition D approximated the pulse duration of condition C by including more cycles in the 7.5 MHz pulses. The three conditions utilized approximately the same duty cycle of about 0.003. The number of different groups to determine the threshold for each exposure condition was limited by setting only 4 values of the peak rarefactional pressure amplitude (PRPA), based on expected thresholds from the previous research.

The pulse parameters were measured and set using a calibrated hydrophone with a 0.2 mm sensitive spot (model HMA-0200, Onda Corp., Sunnyvale, CA). For exposimetry of the ultrasound reaching the lung surface, attenuated pulse parameters were measured by placing samples of chest wall, including skin, muscle and ribs, as close as possible to the

hydrophone and aiming the exposure transducers through an intercostal space. The chest wall samples were obtained from the right side exposure area and were held in a thin plastic bag for testing. The intercostal space was 4–5 mm wide, and the tissue thickness was ~5 mm. The pulse waveforms were recorded and used for calculating the pulse duration, peak rarefactional pressure amplitude (PRPA), the mean of the peak rarefactional and peak compressional amplitudes ($\langle p \rangle$) and the spatial-peak pulse-average (SPPA) intensity according to standard methods (FDA, 2008). The value of attenuated PRPA divided by the square root of the ultrasonic frequency also can be calculated for comparison to the Mechanical Index (MI).

Animal preparation

All *in vivo* animal procedures were conducted with the approval and guidance of the University Committee on Use and Care of Animals, University of Michigan, Ann Arbor, MI. One hundred thirty female Sprague Dawley rats (CD IGS strain, Charles River, Wilmington, MA, USA) were used for this research. Of these, two rats were lost from the study due to anesthetic death, and 10 were excluded due to technical problems. The rats weighed 213 ± 20 gm and each was anesthetized with IP injection of 91 mg/kg ketamine (Ketaved® ketamine hydrochloride injection, Vedco Inc., St. Joseph, MO, USA) plus 9 mg/kg IP xylazine (AnaSed® xylazine injection, Akorn Inc., Decatur, IL, USA). This anesthesia combination has been used for most research on ultrasound induced PCH. These methods duplicate the methods of the previous study of the frequency dependence of PCH thresholds (Miller et al. 2015a).

The right thorax of each rat was shaved and depilated for ultrasound transmission. For exposure, the rats were mounted on a plastic board and aligned vertically in a 38 °C water bath. The water bath maintained the body temperature of the rats and allowed precise aiming of the exposure beam. Five min after exposure, each rat was sacrificed under anesthesia by exsanguination of the inferior vena cava. The trachea was occluded to maintain lung volume and the heart and lungs were removed together. The right cranial and medial lobes, which were the target of the imaging, were then examined and photographed using a stereomicroscope with digital camera (Spot Flex, Diagnostic Instruments Inc., Sterling Heights, MI USA). The photographs of the lungs were used to measure the approximate diameter and area of the region of PCH on the lung surface using image analysis software (Spot v. 5.1, Diagnostic Instruments, Inc., Sterling Heights, MI USA). The area measurement involved freehand outlining of the PCH regions on each lung to include irregularities of the roughly circular regions.

Experimental Plan and Statistics

For each pulsing condition, 4 groups of rats were exposed at different PRPA values. The attenuated PRPA values for the 4 groups of each condition are listed in Table 2. For the highest exposure, listed as group 1, 5 rats were exposed. Groups 2 and 3 had 7 rats for each condition, except for D2 and D3, which had 5 and 6 rats respectively. The lowest group 4 had 5 rats for each condition except for groups B4 and D4, which were limited to 3 rats each because no effect was seen for the higher exposure groups (B3 and D3). In addition, 5 rats

were set up as sham exposures with the aiming step, but no fixed-beam exposure. For each rat, the B mode image was observed, and start and end images were recorded.

Statistical analysis was performed using SigmaPlot for Windows V. 11.0 (Systat Software Inc., San Jose CA, USA). The Student's t-test was used to compare means of two measured parameters, and the Holm-Sidak method was used for multiple comparison. Statistical significance was assumed at $P < 0.05$. In addition, linear regression analysis was used to characterize the variation of PCH area plotted against PRPA and the logarithm of SPPA intensity. The z test of proportions was used to assess the significance of the proportion of rats in each group, which had discernable PCH. The proportion test was used to locate a threshold between the lowest exposure with significant PCH and the next lower-exposure. The uncertainty in this threshold measurement was taken to be equal to the means of the standard deviation of the pulse parameter measurements.

Results

During exposure with the higher PRPAs, a comet tail artifact (CTA) could be seen to grow inward from the lung surface, as shown in Fig. 1 for a group D1 exposure. These artifacts were fixed to the point of exposure on the lung surface, but the tail changed orientation as the transducer and probe assembly was moved, such that the tail was always aligned along the beam-line direction from the imaging probe. All of these fixed-beam exposures resulted in approximately round PCH areas on the lung surface. Examples of the PCH spots are shown in Fig. 2 for a sham exposure and exposures in Groups A2, B1, C2, D1, E2, all of which had exposures with similar PRPAs of 1.2–1.4 MPa, see Table 2. The PCH spots for exposures at 7.5 MHz had somewhat smaller sizes than those at 1.5 MHz.

The results were viewed in two ways. First, in order to show the changes in PCH with exposure, the PCH areas were plotted against PRPA (Fig. 3) and SPPA intensity. By linear regression, the PCH areas increased rapidly with exposure above an intercept. The linear regression included all positive results, which was 2 to 4 points for the different conditions. The intercepts are listed in Table 3 together with the coefficient of determination for the linear regressions. Remarkably, all of the exposure conditions had about the same intercepts of ~ 0.73 MPa, and ~ 20 W/cm².

Second, the proportion of rats with positive results was determined for each group, and the statistical significance of the occurrence determined from the z test of proportions, as listed in Table 2. Thresholds were determined as the mean of the lowest exposure setting with statistically significant occurrence of PCH and the next lower setting, as listed in Table 3 for the PRPA, $\langle p \rangle$, and SPPA intensity. The threshold spatial-peak temporal-average (SPTA) intensities were low: for groups A and B, the duty cycle was 4.12×10^{-4} . The SPTA thresholds, for example, for groups A and B were therefore 8.8 mW/cm² and 7.5 mW/cm², respectively. A multiple pairwise comparison showed that thresholds for conditions A, B and D were not significantly different, and likewise for conditions C and E. However, condition A, B and D thresholds, at about 0.7 MPa, were significantly lower ($P < 0.01$) than condition C and E thresholds, at about 1.15 MPa. This relative difference also was found for statistical comparisons of the thresholds in terms of $\langle p \rangle$ and SPPA intensities (Table 3).

Discussion

Five different pulsed-ultrasound exposure conditions were investigated in terms of PCH area and occurrence (Table 1). For each condition, four groups were exposed for 5 min at a range of PRPA values including the PCH thresholds (Table 2). Groups A and B compared 1.5 MHz to 7.5 MHz with same pulse mode of 10 μ s pulses at 25 ms. The use of identical pulse timing eliminated the increases in PRPA thresholds previously seen for higher frequencies with DUS pulses of shorter duration (Table 4, Miller et al. 2015). These results indicated that the ultrasonic frequency per se did not affect the threshold results, but pulse duration and other timing parameters did. The use of modulated pulse modes, which simulated DUS exposure to some extent, resulted in higher occurrence thresholds (Table 3). The value of PRPA divided by the square root of frequency was 0.98 MPa/MHz^{1/2} at 1.5 MHz and 0.41 MPa/MHz^{1/2} at 7.5 MHz. However, when longer pulses of 12 cycles (1.5 μ s duration at 7.5 MHz) were used, compared to 2 or 3 cycles (0.3 μ s at 7.5 MHz or 1.7 μ s at 1.5 MHz), the threshold was comparable to the non-modulated groups.

When the PCH area results were analyzed by linear regression, all the conditions tested had nearly the same PRPA intercept (Fig. 3), a noteworthy lack of variation for the varied conditions. The 7.5 MHz beam size was much smaller than the 1.5 MHz beam size (Table 1), which resulted in smaller PCH areas at 7.5 MHz (Fig. 2, Table 2). The indication that beam size was important relative to a smaller role for the ultrasonic frequency was also noted by O'Brien et al. (2001). The 7.5 MHz PCH sizes were relatively small, but not as small as might be expected from the beam diameter of 0.83 relative to the 3.8 mm at 1.5 MHz. For example, Group A2 at 1.5 MHz and 1.2 MPa had PCH averaging 3.3 mm in width, while Group B1 at 7.5 MHz and 1.3 MPa had PCH averaging 2.5 mm in width. The relatively larger PCH sizes for the higher frequency, in particular, may have been due to the phenomenon of lung sliding. Some regions of the lung surface moved by 1 mm or more during breathing, so that the beam impacted an area larger than the fixed-beam size.

The results of this study are compared to earlier fixed-beam pulsed ultrasound results from Child et al. (1990), Zachary et al. (2001) and O'Brien et al. (2003) in Table 4. The 1.2 MHz result of Child et al. (1990) for 10 μ s pulses was about the same as our 1.5 MHz result for 10 μ s pulses. However, the higher frequency of 3.7 MHz resulted in a higher threshold (than our 7.5 MHz result), and indicated a small dependence of the thresholds on ultrasonic frequency. The difference between 0.7 MPa at 1.2 MHz and 1.0 MPa at 3.7 MHz is not large for a three-fold frequency increase. The uncertainties in threshold determinations, which include uncertainties in dosimetry, are typically ± 10 –15% for the PRPA (e. g. see Table 3). The threshold for 1 μ s pulse duration was also somewhat higher than our results for relatively short pulses for the modulated exposure conditions, but not greatly different from the DUS results. These differences might be due to the different species or other uncertain factors. Overall, our results and those of Child et al. (1990) 25 years ago might be considered to be in rough agreement.

The threshold results from Zachary et al. (2001) and O'Brien et al. (2003) were substantially higher than our results or those of Child et al. (1990), see Table 4. A key difference in the exposure parameters might be the 10 s exposure duration, compared to 3 min for Child et al.

(1990) and 5 min for this study. An interesting finding was that the mice had somewhat higher thresholds than rats (Zachary et al. 2001). The thresholds at 2.8 MHz and 5.6 MHz were not significantly different for mice or rats. The influence of pulse duration on thresholds was examined by O'Brien et al. (2003) at 2.8 MHz for a range of 1.3 to 11.6 μ s durations, all with a 1 ms pulse interval (i. e. changing duty cycle). The thresholds showed a clear trend of decreased thresholds for increased pulse duration, which was also noted in this study. An interesting comparison is between the 2.0 MPa threshold for 11.6 μ s duration, 1 ms interval and 10 s duration and our 0.75 MPa threshold at 1.5 MHz for 10 μ s duration, 25 ms interval and 300 s duration: the numbers of pulses delivered was comparable at 10,000 and 12,000, respectively. This is interesting because the total on-time was about the same, but the thresholds were substantially different. The higher duty cycle of 0.0116 gives higher SPTA intensity, compared to our 0.0004 duty cycle, and might have been expected to produce a lower, not higher threshold, which may implicate the exposure duration as a key factor. A longer exposure duration may influence the thresholds in some way other than by simply adding more pulses. For example, a relatively slow physiological response to each pulse may gradually accumulate at the lung surface leading to delayed PCH. Further research is needed to test hypotheses such as this and to fully understand the PCH process.

Conclusions

Fixed-beam pulsed ultrasound exposure were compared to simulated diagnostic scanning exposures, and to previous research. Fixed-beam pulsed exposure produced lower thresholds than simulated and actual diagnostic ultrasound (based on occurrence). The total exposure duration may be an unexpectedly important parameter for thresholds. Finally, the thresholds do not appear to depend on ultrasonic frequency per se, but may be higher for higher frequency diagnostic ultrasound due to frequency related changes in pulse-timing parameters.

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Figure 1.

B mode images before and after aiming and exposure: (a) pre-exposure image for aiming, showing the entire right side of the rat thorax with the bright lung surface images shown at about 5 mm from the skin surface, (b) pre-exposure image in the exposure position, (c) post exposure image in the aiming position showing a comet tail artifact oriented directly along the beam direction, (d) post exposure image in the exposure position with a comet tail artifact extending downward away from the point of exposure on the lung surface. The comet tail in (d) appeared and grew longer during the exposure. The scale bar along the left side of the images is in cm.

Figure 2.

Photomicrographs of the PCH areas found on rat lungs for the sham, and each exposure condition (Table 1) using similar PRPA values of 1.2–1.4 MPa. The 1.5 MHz beam (A, C) produced somewhat larger PCH areas than the 7.5 MHz beam (B, D, E) due to the larger beam diameter. The 2 mm scale bar in S applies to all photomicrographs.

Figure 3.

Plots of the PCH areas found for the different PRPA levels for the different exposure conditions (Table 1). The 1.5 MHz exposures (squares) generally produced larger PCH areas than the 7.5 MHz exposures (circles), particularly for the short duration pulses (group E).

Table 1

The parameters used for the 5 exposure conditions.

Condition	Frequency MHz	Pulse Duration n	PRP μs	Repetition ms	Diameter mm	Area mm ²
A	1.5	16	10.3	25	na	3.8
B	7.5	76	10.3	25	na	0.83
C	1.5	3	1.7	0.5	25	3.8
D	7.5	12	1.5	0.5	25	0.83
E	7.5	2	0.3	0.1	25	0.83

The value of n is the number of cycles in the pulses set on the function generator. PRP, pulse repetition period; Repetition, time interval setting of the Gaussian modulation; Diameter and Area, the -6 dB beam size at the focus.

Table 2

Results for each group of the 5 exposure conditions.

Group	PRPA MPa	ISPPA W/cm ²	Width mm	Area mm ²	PCH fraction	z Test P
A1	1.6 ± 0.1	109 ± 16	5.0 ± 1.1	18.4 ± 6.4	5/5	
A2	1.2 ± 0.09	63 ± 9.0	3.3 ± 0.8	11.2 ± 5.0	7/7	
A3	0.87 ± 0.6	28 ± 4.3	1.8 ± 0.9	3.7 ± 1.9	6/7	<0.02
A4	0.63 ± 0.04	14.2 ± 2.2	0.5 ± 0.9	0.6 ± 1.3	1/5	
B1	1.3 ± 0.2	68 ± 25	2.5 ± 0.7	6.8 ± 3.1	5/5	
B2	0.82 ± 0.14	25 ± 8.8	1.1 ± 0.6	1.5 ± 1.1	5/5	
B3	0.55 ± 0.08	10.8 ± 3.3	0	0	0/6	
B4	0.42 ± 0.06	5.8 ± 1.7	0	0	0/3	
C1	1.9 ± 0.1	169 ± 22	3.4 ± 0.6	11.0 ± 1.6	5/5	
C2	1.4 ± 0.08	84 ± 11	2.8 ± 0.6	6.8 ± 1.5	7/7	
C3	1.0 ± 0.06	40 ± 5.2	0.75 ± 1.0	1.3 ± 2.0	3/7	0.31
C4	0.71 ± 0.04	18.0 ± 2.5	0.04 ± 0.10	0.03 ± 0.07	1/5	
D1	1.3 ± 0.2	68 ± 25	2.0 ± 0.9	5.4 ± 3.9	5/5	
D2	0.82 ± 0.14	25 ± 8.8	0.68 ± 0.57	0.61 ± 0.60	5/7	<0.05
D3	0.55 ± 0.08	10.8 ± 3.3	0	0	0/7	
D4	0.42 ± 0.06	5.8 ± 1.7	0	0	0/3	
E1	2.3 ± 0.2	234 ± 82	2.0 ± 0.8	5.2 ± 3.2	5/5	
E2	1.4 ± 0.2	72 ± 24	0.9 ± 0.7	1.9 ± 2.8	6/7	<0.02
E3	0.83 ± 0.10	23 ± 7.1	0.4 ± 0.4	0.3 ± .4	4/7	0.15
E4	0.57 ± 0.07	9.9 ± 2.8	0	0	0/5	

The PRPA is the attenuated peak rarefactional pressure amplitude, and ISPPA is the attenuated spatial-peak pulse- average intensity. The PRPA was the same for groups B and D because these long pulses varied in duration but not in peak amplitude. The z test was relative to 0 PCH in 5 or 7 rats (mostly positives or negatives were not tested).

Table 3

Threshold values of dosimetric parameters based on the zero intercept of linear regression of PCH area against PRPA (Fig. 2) or ISPPA, and on the z test (Table 2), given by the mean of the lowest MI setting with statistically significant occurrence of PCH and the next lower setting: PRPA, Peak rarefactional pressure amplitude; $\langle p \rangle$, the mean of the peak compressional and peak rarefactional pressure amplitudes; I_{sppa} , the spatial peak, pulse average intensity calculated from the pulse waveforms.

Exposure Condition	Linear Regression Intercept				z test	
	PRPA MPa	r^2	I_{sppa} W/cm ²	r^2	PRPA MPa	$\langle p \rangle$ MPa
A	0.65	0.74	16.1	0.72	0.75 ± 0.06	0.81 ± 0.06
B	0.70	0.61	18.6	0.61	0.69 ± 0.11	0.75 ± 0.13
C	0.76	0.87	23.4	0.84	1.20 ± 0.07	1.41 ± 0.1
D	0.76	0.51	22.5	0.51	0.69 ± 0.11	0.75 ± 0.13
E	0.77	0.43	22.6	0.41	1.12 ± 0.15	1.33 ± 0.25
						47.5 ± 15.5

Table 4

A comparison of these results to previously reported findings of in situ PRPA thresholds.

Frequency MHz	Method	Pulse μ s	PRP ms	Frames s ⁻¹	Duration s	PRPA MPa	Reference
1.5	R-PW	10	25	-	300	0.75	This study
7.5	R-PW	10	25	-	300	0.69	
1.5	R-Mod	1.7	0.5	40	300	1.20	
7.5	R-Mod	0.3	0.1	40	300	1.12	
1.5	R-DUS	1.5	0.42	36.4	300	1.03	Miller et al. 2015
4.5	R-DUS	0.39	0.16	32.1	300	1.28	
7.6	R-DUS	0.25	0.10	39.0	300	1.18	
12.0	R-DUS	0.16	0.084	50.8	300	1.36	
1.2	M-PW	10	10	-	180	0.7	Child et al. 1990
3.7	M-PW	10	10	-	180	1.0	
3.7	M-PW	1	1	-	180	1.4	
2.8	M-PW	1.42	1	-	10	3.6	Zachary et al. 2001
5.6	M-PW	1.17	1	-	10	3.0	
2.8	R-PW	1.42	1	-	10	2.3	
5.6	R-PW	1.17	1	-	10	2.8	
2.8	R-PW	1.3	1	-	10	3.1	O'Brien et al. 2003
2.8	R-PW	4.4	1	-	10	2.8	
2.8	R-PW	8.2	1	-	10	2.3	
2.8	R-PW	11.6	1	-	10	2.0	

The methods included rats (R) and mice (M); PW, pulsed wave; Mod, modulated; DUS, diagnostic ultrasound; PRP, pulse repetition period; Frames, frames per s; Duration, total exposure duration including on and off time.