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The Dependence of Thresholds for Pulmonary Capillary Hemorrhage on Diagnostic Ultrasound Frequency

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Abstract

Pulmonary ultrasound examination has become routine for diagnosis in many clinical and point-of-care medical settings. However, the phenomenon of pulmonary capillary hemorrhage (PCH) induction during diagnostic ultrasound imaging presents a poorly understood risk factor. PCH was observed for 1.5 MHz, 4.5 MHz and 12.0 MHz diagnostic ultrasound of anesthetized rats to investigate the frequency dependence of PCH thresholds. PCH was detected in the ultrasound images as growing comet tail artifacts, and assessed using photographs of the surface of excised lungs. Previous photographs acquired with 7.6 MHz diagnostic ultrasound were also included for analysis. In addition, dosimetric parameters were measured at each frequency, including peak rarefactional pressure amplitudes (PRPA) and spatial peak pulse-average (SPPA) intensities attenuated by rat chest-wall samples. PRPA thresholds were determined at each frequency, based on the proportion of PCH occurrence in groups of 5 rats, and were 1.03 ± 0.02 MPa, 1.28 ± 0.14 MPa, 1.18 ± 0.12 MPa and 1.36 ± 0.15 MPa at 1.5, 4.5, 7.6 and 12.0 MHz, respectively. Although the PCH lesions decreased in size with increasing ultrasonic frequency, owing to the smaller beam widths and scan lengths, the PRPA thresholds remained approximately constant. This dependence was different from that of the Mechanical Index, which indicates a need for a specific dosimetric parameter for safety guidance in pulmonary ultrasound.

Keywords

Pulmonary ultrasound; point of care ultrasound; comet tail artifact; bioeffects of ultrasound; pulmonary hemorrhage; Mechanical Index

Introduction

Pulsed ultrasound can induce pulmonary capillary hemorrhage (PCH) in mammals, a bioeffect that was discovered more than 20 yr ago (Child et al. 1990). PCH was

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subsequently studied to determine its dependence on various pulsed-ultrasound parameters using laboratory exposure systems (AIUM, 2000; Church et al. 2008). Only a few studies have utilized actual diagnostic ultrasound (DUS) machines. DUS was used for studies in monkeys (Tarantal and Canfield 1994) and rats (Holland et al. 1996), but the positive results were not clearly definitive. A study of human lungs indicated that echocardiography, which was thought to interact with the lung as incidental exposure, did not induce detectable PCH (Meltzer et al. 1998). Recently, DUS with a small linear array generated PCH in rats, which was detectable as growing comet-tail artifacts in the images and was measured as hemorrhage areas on the lungs (Miller, 2012). These DUS studies have had inconsistent methods and results, which are difficult to compare in order to define PCH thresholds and the dependence of the thresholds on DUS frequency. Nevertheless, these studies have clearly shown that PCH can be induced by diagnostic ultrasound. This finding presents a potential risk of patient injury in pulmonary ultrasound examinations; however, despite a substantial research effort, the risk remains poorly understood.

Several authoritative reviews have been conducted to clarify the dosimetry of PCH, particularly in regard to the utility of the on-screen Mechanical Index (MI) for appraising PCH risk. Laboratory animal studies, which utilized pulsed ultrasound systems to determine thresholds for PCH, were reviewed by the American Institute of Ultrasound in Medicine (AIUM, 2000). The thresholds at ultrasonic frequencies between 1 and 4 MHz were expressed in terms of the pulse peak rarefactional pressure amplitudes (PRPA) derated for tissue attenuation. These results were plotted and a least-squares fitted line approximated the functional form of the MI, with a threshold constant of 0.63 and a frequency exponent of 0.54. For reference, the guideline upper limit for diagnostic ultrasound is $MI = 1.9$ with the 0.5 frequency exponent (FDA, 2008). At that time, cavitation was thought to be involved (Holland et al. 1996) and the MI thus seemed to be a good dosimetric guide for PCH.

These early results were obtained mostly in mice, but ultrasound-induced PCH also has been found at about the same derated PRPAs in mice, rats, rabbits, pigs and monkeys. Another authoritative review organized by the American Institute of Ultrasound in Medicine found no consistently defined risk for diagnostic ultrasound (Church et al. 2008). Ultrasound scanning of patient's lungs at that time was expected mostly from incidental exposure during echocardiography, which would have lower PRPAs than direct exposure. In addition, the study (Meltzer et al. 1998), of human lungs with potential incidental exposure had not found evidence of PCH for 3.5 MHz DUS up to 2.4 MPa ($MI=1.3$). However, direct pulmonary ultrasound examination now has become routine for aiding patient evaluation and diagnosis of 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). This method is well suited to point-of-care settings (Koenig et al. 2011) owing to the small footprint and portability of modern DUS machines. Modern DUS machines also have the on-screen readout of the Mechanical Index (MI), a dosimetric parameter which is specifically related to ultrasonic cavitation and might be useful as a safety guide for pulmonary sonography.

Several studies examined the cavitation hypothesis and found that cavitation was probably not the mechanism for PCH (Raeman, 1997; O'Brien et al. 2000, 2004). This finding

complicated the use of the MI as a safety parameter for PCH. Studies in rats and mice have investigated the influence of specific parameters of pulsed ultrasound in PCH. A meta-analysis of 14 studies found that widely varying thresholds had been reported, which depended on pulse duration, pulse repetition frequency, and exposure duration in addition to PRPA and frequency (Church and O'Brien 2007). In that review, a dosimetric parameter specifically relevant to PCH was derived, which included the pulse duration, pulse repetition frequency, and exposure duration. However, this review depended on data from laboratory pulsed-ultrasound systems with a single fixed beam, rather than from DUS machines.

For the recent DUS study from our laboratory (Miller, 2012), a 7.6 MHz linear array induced PCH in rats scanned for 5 min above a threshold of about $MI = 0.44$ (on-screen readout) for PCH occurrence. This result provided new information at a relatively high diagnostic frequency. However, differences in methods, particularly the use of DUS scanners, make comparisons with previous research using fixed-beam laboratory pulsed-ultrasound systems difficult. The objective of this present research was to extend the use of diagnostic ultrasound methods (Miller, 2012) in order to explore the dependence of thresholds for PCH on DUS frequency, which may help to clarify the risk of PCH for different pulmonary ultrasound examinations. Results at 7.6 MHz were augmented with comparable data from scanning at 1.5, 4.5 and 12.0 MHz with diagnostic ultrasound machines encompassing the commonly used range of diagnostic ultrasound frequencies.

Methods

Animal preparation

All *in vivo* animal procedures were conducted with the approval and guidance of the University Committee on Use and Care of Animals. Female Sprague Dawley rats (CD IGS strain, Charles River, Wilmington, MA, USA) were used for this research. The study progressed in three parts involving 1.5, 4.5 or 12.0 MHz scanning, all with the same general methods. Each rat was weighed and 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. Interestingly, the use of xylazine in this mixture was found to enhance the sensitivity to PCH (Miller et al. 2014), probably as a result of the effects of xylazine on pulmonary physiology (Amouzadeh et al., 1991, 1993). The combination of ketamine and xylazine produced the lowest thresholds for PCH in an investigation of common anesthesia techniques, including ketamine alone, pentobarbital, and isoflurane inhalational anesthesia (Miller et al. 2015). Therefore, the results of this present study presumably represent reasonable worst case conditions, which may encompass patient conditions, illnesses or drug treatments giving enhanced sensitivity to PCH.

The right thorax of each rat was shaved and depilated to allow good ultrasound transmission. The heart rate was checked either with a pulse oximeter (SurgiVet V3395 TPR, Smiths Medical Inc. St Paul, MN USA), or with a laboratory ECG monitoring system (Model ECGA amplifier, Hugo Sachs Elektronik, Harvard Apparatus, March, Germany, with Powerlab 4/30 digitizer and Chart Pro 5, v. 5.5.5 ECG analysis software, ADInstruments

Inc. Colorado Springs, CO USA). The rats were mounted on a plastic board and aligned vertically in a 38 °C water bath for ultrasound scanning as described previously (Miller, 2012). The water bath maintained the body temperature of the rats and allowed consistent scanning. The rats were each sacrificed under anesthesia by exsanguination of the inferior vena cava 5 min after the completion of the procedure. 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 stereo microscope with digital camera (Spot Flex, Diagnostic Instruments Inc., Sterling Heights, MI USA). The photograph of the scanned lung area were used to measure the area of the hemorrhagic region 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 hemorrhage regions on each lung to provide accurate measurements of the irregularly shaped areas. For consistency, this same area measurement method was also applied to photographs from the earlier study (Miller, 2012), which had previously been determined from the product of approximate lengths and widths. The area measurements provide quantitative information about lesion size. The magnitude of the effect can also be described in terms of a volume of hemorrhage or volume of affected tissue. For example, O'Brien et al. (2001) estimated lesion volumes for fixed-beam exposures using measurements of lesion depth in histological sections, combined with lesion surface area (assuming a conical shape). For the scanned DUS exposures, however, the complex lesion volume is difficult to ascertain accurately (e. g. see Miller et al. 2014) and was not measured for this study, which concentrated primarily on threshold determinations.

Ultrasound

Two different ultrasound machines were used with different probes to provide exposures at 1.5 MHz, 4.5 MHz and 12 MHz as listed in Table 1. The 1.5 MHz system was a GE Vingmed System V with FPA2.5 phased array probe (General Electric Corp., Cincinnati OH USA). The face of the phase array was 2.1 cm wide, but the image width of the sector scan depended on the depth (distance from the transducer face). This relatively low frequency probe produced a poor thoracic image of the small rats. The aim of this probe between ribs of the upper thorax was aided by imaging with an FPA10 probe on the same machine, which was operated at 8 MHz with 5 cm depth. A power setting of -20 dB (MI=0.1) was used to avoid potential lung injury from the aiming procedure. The two probes were mounted in parallel on a sliding gantry, so that the probes could be switched as needed and scan the same plane. This aiming procedure helped to assure avoidance of ribs and placement of the lung surface at the desired depth. In addition, the higher resolution image more clearly displayed comet tail artifacts, which were indicative of PCH, after the low frequency scanning. For exposure at 1.5 MHz after aiming, the beam path was blocked, the machine was switched to the FPA2.5 probe with the desired settings and the block was quickly removed to start the scanning.

A GE Vivid 7 ultrasound machine was used for both the 4.5 MHz scanning with a 7L probe, and the 12.0 MHz scanning with an i13L probe. The 7L was a 5 cm long linear array. The i13L was a "hockey stick" linear array probe, which was similar to the 3 cm long linear array probe used for the previous 7.6 MHz scanning (Miller, 2012). The imaging settings are

listed in Table 1 for these probes, together with the settings previously used for 7.6 MHz scanning with a 1.2 cm linear array. Both linear array probes provided images which were satisfactory for aiming. The image width was 4.7 cm for the 7L probe, which covered the entire right side of the rat, while the 1.9 cm image width of the i13L probe only covered part of the thorax. The probes were aimed using -20 dB and -14 dB power settings for the 7L and i13L probes, respectively, and then rapidly switched up to the desired power setting for exposure.

Ultrasonic Exposimetry

The attenuated ultrasonic pulse parameters were measured by placing chest wall samples, including skin, muscle and ribs, between the probes and calibrated hydrophones. The samples of chest wall were obtained from the right side of scanned rats after removal of the lung tissue to specifically include the ultrasound beam path. The chest wall samples were refrigerated and used within two days. For the 1.5 MHz and 4.5 MHz probes, the samples were held in a thin plastic bag which was inserted between the probe and hydrophone, as close as possible to the hydrophone without touching it. The sample bags contained small volumes of sterile saline to eliminate air pockets, and were allowed to warm to the 38 °C water bath temperature before testing. For the 7.6 MHz and 12 MHz probes, which actually touched the skin during the exposure-scanning, the samples were held directly onto the probe surface with plastic wrap. In both situations, the samples were adjusted so that the scan plane of the probe passed between ribs, which closely approximated the configuration during the exposure scanning. The intercostal space was 4–5 mm wide, and the thickness of the intercostal tissue was ~5 mm. For initial tests at 1.5, 4.5 and 7.6 MHz, a calibrated membrane hydrophone with 0.4 mm sensitive spot (model 805, Sonora Medical Systems Inc., Longmont CO USA) was used for the measurements. Later, a newer hydrophone with a 0.2 mm sensitive spot (model HMA-0200, Onda Corp., Sunnyvale, CA) was utilized for measurements of the 12 MHz pulses. The newer hydrophone had the most recent calibration, the smallest spot size and flattest frequency response over the pulse bandwidths. To assure consistent specification of the pulse parameters, the initial measurements were adjusted to conform to the newer hydrophone calibration values. For each exposure setting, the peak rarefactional pressure amplitude (PRPA), the mean of the peak rarefactional and peak compressional amplitudes and the spatial-peak pulse-average (SPPA) intensity were determined. General parameters determined for each probe are listed in Table 2. The approximate attenuation coefficients of 1.1–1.3 dB/cm/MHz, which were derived from the measurements, were commensurate with previous determinations (Teotico et al. 2001). The measurements provide a good estimate of the ultrasound reaching the lung for the different probes, but it should be noted that interaction with the lung itself, particularly the tissue gas interface, greatly modifies the in situ parameters. The exact in situ dosimetry depends on the conditions at the lung surface (O'Brien et al. 2002; Oelze et al. 2008). Unfortunately, measurements at the tissue-gas interface would be impossible with present state-of-the-art hydrophones.

Experimental Plan and Statistics

For each frequency, 5 individual rats were scanned at each specific power setting. The power setting included the maximum available, which was shown as 0 dB on the ultrasound

machines. Lower power settings were located at -2 dB intervals, with the minimum setting established at the highest power setting with no evident PCH. In addition, sham-exposed animals were prepared for scanning, including the aiming steps, but then not scanned above the very low power settings (-14 to -20 dB) used for aiming. The power settings are reported here as the corresponding MI readout (only the MI readout was available for the previous tests at 7.6 MHz). For 1.5 MHz the MI readout ranged from 1.7 to 0.5 in six steps. At 4.5 MHz there were 6 steps ranging from MIs of 1.2 to 0.4. At 12.0 MHz, only 4 steps were needed, ranging from MIs of 1.2 to 0.6. The previous work at 7.6 MHz involved 5 steps from MIs of 0.9 to 0.27. For each rat, starting and ending images were recorded and the comet tail artifacts (CTAs) were assessed if possible as a percentage of the bright lung surface image. In addition, when possible, the time to perception of CTAs was noted. The length, width and area of the PCH regions on the lungs were determined for each rat.

Statistical analysis was performed using SigmaPlot for Windows V. 11.0 (Systat Software Inc., San Jose CA, USA). The Mann-Whitney rank sum test was used to compare means of the measured parameters, with statistical significance assumed at $P < 0.05$. The z test of proportions was used to assess the significance of the proportion of 5 rats, which had pulmonary hemorrhage, for each group scanned at a specific MI. The proportion test was used to locate a threshold between the lowest MI with significant PCH and the next lower MI setting. The uncertainty in this threshold measurement was taken to be equal to plus or minus one standard deviation of the pulse parameter measurements, which neglects the uncertainty related to the step size (± 1 dB or about $\pm 12\%$ of the pressure threshold). In addition, regression analysis was used to fit the data means plotted against PRPA, and to gauge the influence of the ultrasonic frequency on the threshold results.

Results

Images at the start and end of a 1.5 MHz exposure scan at $MI = 1.4$ (-2 dB, or $PRPA = 1.8$ MPa), are shown in Fig. 1. There is a discernable comet tail artifact in the end-image, but the images are generally poor. Other scans, which produced PCH, did not have any indications of CTAs. The 8 MHz images (for aiming) are shown in Fig. 2 and the post exposure scan image had clear CTAs. However, these images were also of poor quality, owing to the -20 dB power setting; therefore, the CTA lengths in the lung surface images were not determined for the 1.5 MHz tests. An example of the PCH region induced by 1.5 MHz scanning is shown in Fig. 3 for $MI = 1.4$ (1.8 MPa PRPA). For this frequency the PCH was substantial for the 0 dB setting ($MI = 1.7$, 2.2 MPa PRPA), as shown in Fig. 4. PCH extended across the entire medial lobe and included portions of the cranial and caudal lobes. The posterior side of the medial lobe had blood filled regions, which indicated the pulmonary capillary injury through the entire lobe (although the extent of these blood filled regions suggests that the PCH had spread partly by flowing through airways). The results for all the 1.5 MHz tests are listed in Table 3, with the threshold indicated between the $MI = 0.5$ and 0.7 settings.

Fig. 5 shows the start and end images, and the resulting PCH for 4.5 MHz scanning at $MI = 0.8$ (1.8 MPa PRPA). This relatively wide image includes the entire thorax and front legs. CTAs spread across the entire bright lung surface image, and the PCH extended across the

medial lobe. The gap in the CTA pattern corresponds to a gap in the PCH, which was likely due to the presence of a rib in the image plane. The PCH sometimes extended through the affected lobe, as shown in Fig. 6 for the cranial lobe of a lung scanned at MI=1.2 (2.8 MPa PRPA). The data for the 4.5 MHz scanning is listed in Table 4, with the threshold indicated between the MI=0.5 and 0.6 settings.

Fig. 7 shows a presentation similar to Fig. 5, but for the 12 MHz scanning at the MI=1.2 setting (1.8 MPa PRPA). The crisp, high resolution images show extensive CTAs, which correspond to the region of PCH. However, the size of the PCH region in Fig. 7 was smaller than the effects shown in Fig. 5. The results for 12 MHz scanning are listed in Table 5, with the threshold indicated between the MI=0.8 and 1.0 settings.

For comparison, results for the previous 7.6 MHz scanning were given in Table 1 of Miller (2012). Exposure parameters are also listed in Tables 1 and 2. The occurrence threshold for that study was located between the MI=0.37 and 0.52 settings using the Z test.

The varying magnitudes of the PCH effects can be portrayed by the area of PCH found on the lung surface, as shown in Fig. 8 plotted versus the PRPA. For Fig. 8 the new measurement of area and attenuated PRPA were used for the 7.6 MHz data. The two wide-scan probes at the lower frequencies produce a much larger impact than the small higher frequency probes. However, the thresholds were not greatly different. The threshold for each frequency was estimated by performing linear regression on each data point which had a non-zero value (i. e. at least one rat had evident PCH). The results of the linear regression are plotted in Fig. 8 for the PRPA and listed in Table 6 for both the PRPA and the SPTA intensity. For the PRPA thresholds, logarithmic regressions against frequency (i. e. logarithm of thresholds versus logarithm of frequency) yielded an approximate functional dependence of the intercept threshold PRPAs in MPa equal to $0.99f^{0.06}$, in which f is the ultrasonic frequency (e. g. 1 MPa at 1 MHz). For the SPPA intensity, the thresholds in W/cm^2 were approximately equal to $29.5f^{0.23}$ (e. g. $29.5 \text{ W}/\text{cm}^2$ at 1 MHz). These results indicate no, or very weak dependence on frequency across the diagnostic ultrasound range.

The determination of thresholds by the statistically significant occurrence of the PCH at each frequency (see tables 3–5) seems to be a better method than the linear regression method used for Figure 8, because the occurrence parameter minimizes the influence of probe size on the thresholds. The occurrence thresholds are given in Table 7 for the PRPA, mean pressure amplitude and SPPA intensity all with standard deviations determined by the variation in the hydrophone measurements with different chest wall samples. The trends in threshold with frequency from this method of threshold determination also were evaluated by logarithmic regression. The PRPA thresholds and the SPPA intensity thresholds using the significant occurrence method are plotted using logarithmic scaling in Fig. 9. For the PRPA thresholds, the approximate functional dependence of the threshold PRPAs in MPa was equal to $1.0f^{0.12}$ (e. g. 1 MPa at 1 MHz). For the SPPA intensity, the thresholds in W/cm^2 were approximately equal to $32f^{0.37}$ (e. g. $32 \text{ W}/\text{cm}^2$ at 1 MHz). These results again indicate very weak dependence on frequency across the diagnostic ultrasound range.

Discussion

In this study, the influence of different frequencies of diagnostic ultrasound on PCH induction was investigated to extend previous findings at 7.6 MHz (Miller, 2012). Groups of 5 rats were scanned for 5 min at specific power settings using three different ultrasound probes at 1.5 MHz, 4.5 MHz and 12.0 MHz. The pulsed ultrasound parameters were measured after passage through chest wall samples to approximate the ultrasound exposure reaching the lung surface. PCH was observed using comet tail artifacts in the ultrasound images and measured on the surface of the lung post-scanning. The primary endpoint was the PCH threshold of exposure at each frequency. These were estimated from the intercepts of the linear regression of the area of PCH on the lung surface (e. g. see Fig. 8) and are listed for PRPA and SPPA intensity in Table 6. Thresholds were also estimated by determining the mean of the exposure level with a significant occurrence of PCH, and the next lower level, which are listed in Table 7 for the PRPA, PRPA divided by the square root of the frequency, the mean pressure amplitude, and the SPPA intensity. The occurrence method was thought to be somewhat more definitive than the regression method, because it reduced the problems of the non-constant scanned areas and pulse durations, and of the hit-or miss occurrence. Overall there was little dependence of the thresholds on frequency using either method. In particular, the thresholds were less dependent on DUS frequency than the MI.

The best dosimetric parameter for PCH remains uncertain. The parameter developed for cavitation bioeffects is the MI, which is proportional to the PRPA for a given frequency (FDA, 2008). For example, the PRPA is the key parameter for cavitation bioeffects associated with ultrasound contrast agents (Dalecki et al. 2000). Although cavitation initially was considered to be a good candidate for the physical mechanism of PCH (Holland et al. 1996), subsequent research did not support this hypothesis. The use of ultrasound contrast agents to provide cavitation nuclei did not enhance PCH (Raeman et al. 1997; O'Brien et al. 2004). Furthermore, elevated hydrostatic pressure, a classic test for the cavitation mechanism, did not inhibit the production of PCH (O'Brien et al. 2000).

In early research on PCH, the peak compressional pressure amplitude (PCPA) appeared to be equal in importance to the PRPA (Bailey et al. 1996). The PRPA and PCPA are equal for low amplitude (linear propagation) conditions, but for nonlinear conditions the PCPA increases for increasingly non-linear (non-sinusoidal) pulse shapes. This circumstance suggests that the peak mean pressure amplitude (PMPA) might be of more value for the PCH bioeffects than just PRPA, which was the rationale for listing this parameter in Table 7. Frizzell et al. (2003) also noted that the pulse polarity was not critical for PCH magnitude and that the pulse intensity integral, which incorporates both positive-pressure and negative-pressure portions of the pulse, correlated better with the effect than the PRPA. The SPPA intensity, listed in Table 7, is given by the pulse intensity integral divided by the pulse duration. For this study, the threshold pulse intensity integral ranged from $53 \mu\text{W s cm}^{-2}$ at 1.5 MHz to $12.5 \mu\text{W s cm}^{-2}$ at 12.0 MHz, which does seem to reflect the reduced PCH areas for the higher frequencies (Fig. 8). More research will be needed to distinguish the relative influence of different in scanned area and pulse intensity integral on PCH magnitude.

The MI is the dosimetric parameter which is available to sonographers during imaging. This parameter has a detailed definition (FDA, 2008), but essentially estimates the maximum in situ value of the PRPA divided by the square-root of frequency by derating measurements of PRPA in water with an attenuation coefficient of 0.3 dB/cm/MHz. In a comprehensive review of PCH induced by pulsed ultrasound in mice and rats, the in situ thresholds in the 1 – 4 MHz range could be approximately fitted with a line having a frequency exponent of 0.54; that is, much like the MI frequency dependence (AIUM, 2000). However, since PCH does not appear to be due to cavitation, a dependence on MI is not expected. O'Brien et al. (2001) noted that beam width was a much more important factor for the magnitude of the bioeffects than was the frequency. This same trend is evident in Fig. 8 for the different scan-plane thicknesses (Table 2, approximately equivalent to fixed-beam width) at the different frequencies. The PCH surface area (but not the threshold) of the effect is strongly dependent on ultrasonic frequency, likely owing to the smaller scan-plane thicknesses and scan lengths (or to the lower pulse intensity integrals, noted above) at the higher frequencies.

An analysis of the role of various ultrasonic pulse parameters on PCH was conducted by Church and O'Brien (2007) in an effort to develop a better safety index than the MI. For the fixed beam exposures (non-diagnostic ultrasound), frequency, pulse duration, pulse repetition frequency and exposure duration were considered, and predictive equations were developed. This analysis is difficult to relate to this present study using diagnostic ultrasound, because of the scanning at different rates of frames per second was not available for consideration in the laboratory fixed-beam studies. If the pulse repetition frequencies from this study are used, predictive values are much too high. The frame rate for B mode ultrasound might be a reasonable substitute for the pulse repetition frequency in Eq. 6 (Church and O'Brien, 2007). This yields threshold PRPAs from 0.88 MPa at 1.5 MHz to 2.8 MPa at 12.0 MHz. These predicted values have frequency dependence similar to the MI and do not accurately reproduce the PRPA thresholds in this study. Thresholds expressed in terms of the PRPA divided by the square-root of the frequency (Fig. 9, Table 7) decreased from 0.84 at 1.5 MHz to 0.39 at 12 MHz. This divergence indicates that the substitution of frame rate for PRF is not appropriate or, possibly, that the B mode threshold data reported here has a different dependence on parameters from the earlier work with fixed beam pulsed ultrasound.

In conclusion, the dependence of diagnostic ultrasound induced PCH was investigated in the range of 1.5–12.0 MHz. The magnitude of PCH decreased with increasing frequency in accord with the decreasing scan area dimensions. However, the PRPA threshold was essentially constant with frequency, and only a small dependence was noted for the SPPA intensity thresholds. The best dosimetric parameter remains somewhat uncertain, but the PRPA, PMPA or I_{SPPA} appear to be more closely associated with of the PCH thresholds found in this study than the MI.

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References

- American Institute of Ultrasound in Medicine. Section 4--bioeffects in tissues with gas bodies. *J Ultrasound Med*. 2000; 19:97–108. 154–68. [no authors listed]. [PubMed: 10680616]
- Amouzadeh HR, Sangiah S, Qualls CW Jr, Cowell RL, Mauromoustakos A. Xylazine-induced pulmonary edema in rats. *Toxicol Appl Pharmacol*. 1991; 108:417–27. [PubMed: 1902333]
- Amouzadeh HR, Qualls CW Jr, Wyckoff JH 3rd, Dzata GK, Sangiah S, Mauromoustakos A, Stein LE. Biochemical and morphological alterations in xylazine-induced pulmonary edema. *Toxicol Pathol*. 1993; 21:562–71. [PubMed: 8052803]
- Bailey MR, Dalecki D, Child SZ, Raeman CH, Penney DP, Blackstock DT, Carstensen EL. Bioeffects of positive and negative acoustic pressures in vivo. *J Acoust Soc Am*. 1996; 100:3941–6. [PubMed: 8969491]
- Child SZ, Hartman CL, Schery LA, Carstensen EL. Lung damage from exposure to pulsed ultrasound. *Ultrasound Med Biol*. 1990; 16:817–25. [PubMed: 2095012]
- Church CC, O'Brien WD Jr. Evaluation of the threshold for lung hemorrhage by diagnostic ultrasound and a proposed new safety index. *Ultrasound Med Biol*. 2007; 33:810–8. [PubMed: 17383801]
- Church CC, Carstensen EL, Nyborg WL, Carson PL, Frizzell LA, Bailey MR. The risk of exposure to diagnostic ultrasound in postnatal subjects: nonthermal mechanisms. *J Ultrasound Med*. 2008; 27:565–92. [PubMed: 18359909]
- Dalecki D, Child SZ, Raeman CH, Xing C, Gracewski S, Carstensen EL. Bioeffects of positive and negative acoustic pressures in mice infused with microbubbles. *Ultrasound Med Biol*. 2000; 26:1327–32. [PubMed: 11120371]
- FDA. Information for manufacturers seeking marketing clearance of diagnostic ultrasound systems and transducers. Rockville MD: Food and Drug Administration, Center for Devices and Radiological Health; 2008.
- Frizzell LA, Zachary JF, O'Brien WD Jr. Effect of pulse polarity and energy on ultrasound-induced lung hemorrhage in adult rats. *J Acoust Soc Am*. 2003; 113:2912–8. [PubMed: 12765408]
- Holland CK, Deng CX, Apfel RE, Alderman JL, Fernandez LA, Taylor KJ. Direct evidence of cavitation in vivo from diagnostic ultrasound. *Ultrasound Med Biol*. 1996; 22:917–25. [PubMed: 8923710]
- Koenig SJ, Narasimhan M, Mayo PH. Thoracic ultrasonography for the pulmonary specialist. *Chest*. 2011; 140:1332–41. [PubMed: 22045878]
- Meltzer RS, Adsumelli R, Risher WH, Hicks GL Jr, Stern DH, Shah PM, Wojtczak JA, Lustik SJ, Gayeski TE, Shapiro JR, Carstensen EL. Lack of lung hemorrhage in humans after intraoperative transesophageal echocardiography with ultrasound exposure conditions similar to those causing lung hemorrhage in laboratory animals. *J Am Soc Echocardiogr*. 1998; 11:57–60. [PubMed: 9487470]
- Miller DL. Induction of pulmonary hemorrhage in rats during diagnostic ultrasound. *Ultrasound Med Biol*. 2012; 38:1476–1482. [PubMed: 22698500]
- Miller DL, Suresh MV, Dou C, Yu B, Raghavendran K. Characterization of ultrasound-induced pulmonary capillary hemorrhage in rats. *Microvasc Res*. 2014; 93:42–5. [PubMed: 24583360]
- Miller DL, Dou C, Raghavendran K. Anesthetic techniques influence the induction of pulmonary capillary hemorrhage during diagnostic ultrasound in rats. *J Ultras Med*. 2015; 34:289–297.
- O'Brien WD Jr, Frizzell LA, Weigel RM, Zachary JF. Ultrasound-induced lung hemorrhage is not caused by inertial cavitation. *J Acoust Soc Am*. 2000; 108:1290–7. [PubMed: 11008829]
- O'Brien WD Jr, Simpson DG, Frizzell LA, Zachary JF. Superthreshold behavior and threshold estimates of ultrasound-induced lung hemorrhage in adult rats: role of beamwidth. *IEEE Trans Ultrason Ferroelectr Freq Control*. 2001; 48:1695–705. [PubMed: 11800133]
- O'Brien WD Jr, Kramer JM, Waldrop TG, Frizzell LA, Miller RJ, Blue JP, Zachary JF. Ultrasound-induced lung hemorrhage: role of acoustic boundary conditions at the pleural surface. *J Acoust Soc Am*. 2002; 111:1102–1109. [PubMed: 11863166]
- O'Brien WD Jr, Simpson DG, Frizzell LA, Zachary JF. Effect of contrast agent on the incidence and magnitude of ultrasound-induced lung hemorrhage in rats. *Echocardiography*. 2004; 21:417–22. [PubMed: 15209720]

- Oelze ML, Miller RJ, Blue JP Jr, Zachary JF, O'Brien WD Jr. Estimation of the acoustic impedance of lung versus level of inflation for different species and ages of animals. *J Acoust Soc Am*. 2008; 124:2340–2352. [PubMed: 19062872]
- Raeman CH, Dalecki D, Child SZ, Meltzer RS, Carstensen EL. Albuterol does not increase the sensitivity of the lung to pulsed ultrasound. *Echocardiography*. 1997; 14:553–558. [PubMed: 11174994]
- Sartori S, Tombesi P. Emerging roles for transthoracic ultrasonography in pleuropulmonary pathology. *World J Radiol*. 2010; 2:83–90. [PubMed: 21160921]
- Tarantal AF, Canfield DR. Ultrasound-induced lung hemorrhage in the monkey. *Ultrasound Med Biol*. 1994; 20:65–72. [PubMed: 8197628]
- Teotico GA, Miller RJ, Frizzell LA, Zachary JF, O'Brien WD Jr. Attenuation coefficient estimates of mouse and rat chest wall. *IEEE Trans Ultrason Ferroelectr Freq Control*. 2001; 48:593–601. [PubMed: 11370373]

Figure 1. Images obtained for 1.5 MHz scanning at the start (top) and end (bottom). The oval structure was the entire rat thorax with poor resolution of internal structures. Even the bright surface echoes from the lung was difficult to discern. The bottom image has some indication of a comet tail artifact slightly right of center.

Figure 2. Images obtained before (top) and after (bottom) 1.5 MHz scanning using an 8 MHz probe at a low power setting. The bright echoes from the lung surface are evident, and comet tail artifacts are clearly shown in the bottom image (note that these images are reversed right to left from those in Fig. 1).

Figure 3.
The lung sample from the scanning shown in Fig. 1 and 2. A substantial line of PCH spreads across the medial lobe and extends to part of the caudal lobe. Scale bar: 5 mm.

Figure 4.

An example of PCH induced by 1.5 MHz scanning at the maximum power setting viewed from the anterior side (top) and posterior side (bottom). The wide PCH band on the medial lobe extends throughout much of the lobe, and is evident on the posterior side, apparently spreading beyond the scan plane. Scale bar: 5 mm.

Figure 5.

Images at 4.5 MHz before (left, top) and after (left, bottom) scanning with the resulting PCH shown on the right (scale bare: 5 mm). The ultrasound images show the entire cross-section of the rat thorax with the bright lung surface images shown at about 5 mm from the skin surface. The image taken at the end of scanning shows comet tail artifacts extending across the entire surface image, except for a shadowed gap which was likely due to a rib. The PCH on the medial lobe shows substantial hemorrhage, with a gap corresponding to the rib shadow.

Figure 6. Photographs of a lung scanned at the maximum power setting at 4.5 MHz showing the anterior (top) and posterior (bottom) side of the cranial lobe. The collective PCH seems to spread into areas outside the scan plane. Scale bar: 5 mm.

Figure 7.

Images at 12.0 MHz before (left, top) and after (left, bottom) scanning with the resulting PCH shown on the right (scale bare: 2 mm). The ultrasound images show a portion of the rat thorax with the bright lung surface images shown at about 5 mm from the skin surface. At the end of scanning, comet tail artifacts extended across the entire lung surface image, which corresponded to the PCH seen on the lung surface.

Figure 8.

The data for the mean area, with standard error bars, of the PCH seen on the lung surface. Linear regression was used to fit the non-zero means (i. e. including all groups with at least one positive result). The PCH increases rapidly above an apparent threshold (indicated by the intercept with the abscissa), which is about the same for each frequency; however, the magnitude of the of the PCH areas were larger for the larger, lower-frequency probes.

Figure 9.

Logarithmic plots of the PRPA thresholds (top) and SPPA intensity thresholds (bottom) for the occurrence of PCH as a function of diagnostic ultrasound frequency. The data are means of the hydrophone measurements after chest wall attenuation with standard error bars. Linear regressions (solid) with 95% confidence intervals (dashed) indicate little or no frequency dependence for the PRPA thresholds, but small increases in the SPPA intensity thresholds with frequency. The top panel includes an illustration of the square-root frequency dependence of the MI, with the constant equal to $0.56 \text{ MPa/MHz}^{0.5}$. The frequency dependence of the MI would yield a line approximately proportional to frequency (slope equal to one) in the lower panel (this was omitted from the figure, because the definition of the MI does not involve intensity).

A list of the diagnostic ultrasound scan settings for each probe used for imaging-exposure. The frequencies listed were the center frequency of the pulses as measured with a hydrophone, which were obtained with on-screen settings of 1.5 (FPA2.5), 4.0 (7L) and 14 MHz (i13L). The settings for the previously employed 7.6 MHz system (Miller, 2012), which did not show the frequency on screen, are also listed for comparison.

Table 1

System	Probe	Frequency MHz	Image Depth cm	Focal Depth cm	Lung Depth cm	Frame Rate s ⁻¹
GE Vingmed	FPA2.5	1.5	6	5	3.75	36.4
GE Vivid 7	7L	4.5	3	1.3	1.0	32.1
Philips HDI	CI15-7	7.6	2.0	1.0	0.6	39.0
GE Vivid 7	i13L	12.0	1.5	0.65	0.6	50.8

Table 2

General pulse parameters and attenuation determined by hydrophone measurements using the lowest power settings, which approximated linear propagation conditions. The -6 dB thickness of the beam perpendicular to the scan plane was determined as the width at half the maximum pressure amplitude. The approximate attenuation coefficients were calculated from the spatial peak pulse average intensity values for a 5 mm tissue thickness.

System	Probe	Frequency MHz	Pulse Interval μ s	Pulse Duration ns	- 6 dB Thickness mm	Attenuation Coefficient dB/cm/MHz
GE Vingmed	FPA2.5	1.5	420	1510	4.30	1.1
GE Vivid 7	7L	4.5	162	390	3.75	1.1
Philips HDI	CI15-7	7.6	100	250	1.05	1.3
GE Vivid 7	i13L	12.0	84	160	0.75	1.2

Table 3

Results for each group scanned at 1.5 MHz, for which the length of the bright lung surface image and the length showing comet tail artifacts was not available. The Z test was relative to 0 PCH in 5 shams.

Setting MI	PCH Length mm	PCH Width mm	PCH fraction	Z test P
1.7	28.6±8.1	4.2±1.3	5/5	<0.01
1.4	22.7±5.9	3.6±0.7	5/5	<0.01
1.1	17.4±4.2	2.4±0.8	5/5	<0.01
0.9	7.2±5.3	1.4±0.9	5/5	<0.01
0.7	3.2±2.7	0.7±0.5	5/5	<0.01
0.5	0.2	0.2	1/5	>0.05

Table 4

Results for each group scanned at 4.5 MHz. The Z test was relative to 0 PCH in 5 rats at the lowest setting.

Setting MI	Time to Perceptions	Image Length mm	CTA Length percent	PCH Length mm	PCH Width mm	PCH fraction	Z Test P
1.2	16±9	15.6±2.9	100	17.4±4.5	4.9±1.1	5/5	<0.01
1.0	22±14	14.4±2.7	89±12	12.2±2.7	4.2±1.2	5/5	<0.01
0.8	50±52	13.4±2.4	80±20	12.3±3.8	2.9±0.8	5/5	<0.01
0.6	200±105	9.9±2.0	22±25	2.7±2.5	1.1±1.0	4/5	0.01
0.5	-	9.4±3.0	-	-	-	0/5	-
0.4	-	10.7±2.7	-	-	-	0/5	-

Table 5

Results for each group scanned at 12.0 MHz. The Z test was relative to 0 PCH in 5 shams.

Setting MI	Time to Perceptions	Image Length mm	CTA Length percent	PCH Length mm	PCH Width mm	PCH fraction	Z Test P
1.2	5±4.6	7.1±1.3	82±18	5.1±1.0	1.2±0.5	5/5	<0.01
1.0	61±68	7.6±3.1	43±43	2.5±2.5	0.5±0.4	4/5	0.01
0.8	30	6.3±0.8	1.7±2.4	1.5±2.2	0.4±0.5	2/5	>0.05
0.6	-	6.9±1.2	3.9	1.5	0.5	1/5	>0.05

Table 6

Thresholds based on the linear regression for data on PCH areas for the PRPA exposure parameter, which is shown in Fig. 8, and similarly for the SPPA intensity. The threshold is taken to be the intercept of a linear regression with the PRPA axis with 95% confidence intervals (C. I.) and coefficient of determination (r^2).

Frequency MHz	PRPA regressions			ISPPA Regressions		
	Intercept MPa	95% C. I. MPa	r^2	Intercept W/cm ²	95% C. I. W/cm ²	r^2
1.5	0.98	0.86–1.06	0.89	32	25–36	0.85
4.5	1.22	0.98–1.38	0.80	46	34–56	0.78
7.6	1.01	0.70–1.20	0.70	43	30–55	0.70
12.0	1.17	0.66–1.32	0.50	55	24–70	0.49

Table 7

Threshold values of dosimetric parameters for the mean of the lowest MI setting with statistically significant occurrence of PCH and the next lower setting: p_- , Peak rarefactional pressure amplitude; p_-/f , division by the square-root of frequency for comparison to the Mechanical Index; $\langle p \rangle$, the mean of the peak positive and peak rarefactional pressure amplitudes; I_{sppa} , the spatial peak, pulse average intensity calculated from the pulse waveform. Values are given as the mean, plus/minus one standard deviation, which was often comparable to one half the step between the values at the two MI settings.

Frequency MHz	p_- MPa	p_-/f MPa/MHz ^{1/2}	$\langle p \rangle$ MPa	I_{sppa} W cm ⁻²
1.5	1.03 ± 0.02	0.84 ± 0.02	1.16 ± 0.02	35 ± 2
4.5	1.28 ± 0.14	0.60 ± 0.07	1.31 ± 0.08	49 ± 8
7.6	1.18 ± 0.12	0.43 ± 0.04	1.35 ± 0.11	58 ± 10
12.0	1.36 ± 0.15	0.39 ± 0.04	1.78 ± 0.23	78 ± 18